Health, Communities, Disability Services and Domestic and Family Violence Prevention Comr

From: [redacted]
Sent: Monday, 7 January 2019 4:55 PM
To: Health, Communities, Disability Services and Domestic and Family Violence Prevention Committee
Subject: Sub 033 Subsmissions to Queensland Parliament Opposing the Re-Scheduling of Cannabis and / or Cannabinoids
Attachments: 03 FDA Federal Register Submission for WHO Review and Consideration - Genotoxicity & Teratogenicity, Concise 2.pdf; Single Page Lay Summary - Cannabis Genotoxicity and Teratogenicity.pdf; 01 FDA Federal Register Submission for WHO Review and Consideration - On Cannabis Genotoxicity, General.pdf; Cannabidiol and Cannabidivarin cause DNA damage and chromosomal aberrations.pdf; Selected Birth Defects w Prenatal Drug Use - Cannabis, Hawaii, Forrester, 2007.pdf
Importance: High
Categories: Submission

Mr Stephen Miles,
The Minister of Health,
Queensland Government.

Dear Hon. Mr Miles,

I am advised that under pressure from the popular press, the Queensland Government is presently considering re-scheduling and down-scheduling the protections for the public against cannabis.

In my view this would be a grave error.

Many sensible and cautious people have advocated that the introduction of an cannabinoid as a therapeutic agent should follow similar precautions to those for any other proposed medicine. They argue: *"Why should cannabinoids / and or cannabis itself be any different from any other medicine which is required to undergo formal and careful tests to satisfy drug regulators?"*

I agree of course that proper formal and duly conducted clinical trials are required before any medicine is introduced to widespread use.

The Government would I am sure be well aware that the medicalization of cannabis and or cannabinoids overseas has been nothing more than a ruse for the full legalization of cannabis and cannabinoids which generally follows very rapidly thereafter.

However clinical trials do NOT establish the long term safety of drugs, as they are usually conducted over only a few months.

Secondly tests for genotoxicity need to be very carefully conducted outside of clinical trials.

I note that the registered prescribing information for both "Epidiolex" (cannabidiol) and "Sativex", the THC / cannabidiol mixture carry strong warnings relating to genotoxicity.

I was appalled learn recently that Atrial Septal Defect (ASD) has sky-rocketed in many jurisdictions where cannabis is widely used including Arizona, Colorado, Hawaii and Kentucky. Indeed, as shown below these defects are rising sharply, even when shown on a logarithmic plot. ASD has previously been linked with cannabis in a large Hawaiian study – and now we see it showing up on US national neonatal teratology figures!!! Please see graphs below.

Indeed modelling this relationship indicates that in Utah, where cannabis use was recently only 3%, one would expect a predicted ASD rate of only 19.83 / 10,000 live births, whereas in Colorado amongst young people 18-25 years of age where past month cannabis use was recently found to be 32% could expected a modelled ASD rate of 211.52 / 10, 000 live births, which represents a 1,067% elevation in the respective rates.

We are naturally preparing these observations for publication in the peer reviewed literature.

One notes that autism is growing exponentially in every American jurisdiction where it is being measured. Cannabis has been previously linked with these changes in the brain by damaging the brain development during pregnancy, neonatal life and growth and development until at least the early 20's. Whilst it has not been proven that cannabis is the proximate or major cause for this alarming state of affairs, it is the most obvious – and indeed known – environmental neurotoxin and neuroteratogen.

Similarly in the north of California where the cannabis industry is presently burgeoning, a revival of gastroschisis and autism has been documented.

Indeed our letter to JAMA surgery on just this these will be published in the next few weeks.

I draw your attention to the attached paper which recently demonstrated that the known heavy epigenetic footprint of cannabis on the epigenome, which controls gene expression and regulation during both developmental and adult life, has been documented to be damaged in both humans and rats, and in similar ways. The neurobehavioural and neurotoxicological changes observed in humans and mice are virtually identical, as cannabis is known to control almost every major step in brain development.

Documented evidence of extensive damage to the epigenome of human and rodent sperm even prior to fertilization necessarily implies that the human eggs and sperm are prematurely aged even prior to fertilization!!! The implications of this are horrific for any health system!!!

I have been asked to write an opinion piece on this issue recently for a major international epigenomics journal which will hopefully also soon be made available.

Indeed a simple internet search will readily demonstrate that Colorado lawmakers are considering declaring a state of emergency there in relation to the autism epidemic currently growing at a whopping 30% each two years!

There is much more to say on the issue of the genotoxicity of cannabis and cannabinoids.

THE MOST IMPORTANT POINT HOWEVER IS THAT GENOTOXICITY IS A FUNCTION OF MANY OF THE MOST COMMON CANNABINOIDS IN CANNABIS, AND IS NOT LIMITED SIMPLY TO THC ITSELF.

Regulations which seek to control the THC content of hemp, as was recently done in the USA by the Farm Act, reveal a very serious shortcoming of the understanding of both the potency of cannabinoids and the developmental neurobiology, and the profound role of cannabinoids in perturbing the epigenome.

I am working hard in the area of neonatal epidemiology to delineate these various trends more fully in the area of neonatal epidemiology.

I have every expectation that in the coming months we will be submitting major research pieces to leading international medical journals on these subjects.

The point about **Atrial Septal Defect of course is that it has not been previously been understood to have been a clearly cannabis related teratogenic outcome**. However, with documented epidemics of ASD in Kentucky, Hawaii, Colorado and Alaska, all places where the cannabis industry is busy or growing rapidly, there can really be little doubt about the importance of the association. You would I am sure be interested to learnt that the Hawaiian study indicated an increased risk of 6-fold, with a confidence interval range from 2-14 times background.

Paradoxically, the USA Farm bill, which was supposed to bring relief to the hard hit farmers of Kentucky by allowing them to grow hemp to replace tobacco, may actually the cause of their undoing with the Kentucky ASD rate actually "going vertical" in all probability from this cause. Indeed the internet tell me that a 500 acre hemp farm is now planned for Kentucky – based by Australian investor dollars!

Indeed the question of the hour must surely be: ***"How many more defects will turn out to be cannabis related?"***

You may consider the obvious close relationship between cannabis consumption and the chromosomal trisomies including Down's syndrome shown in the maps below. This was also found in Hawaii.

USA's CDC have twice stated that cannabis causes anencephaly, babies born without brains and mostly do not live very long (less than one hour). **However in linking cannabis with Downs syndrome and atrial septal defect these are some of the commonest of all defects – which cannabis make much more common.**

For indeed cannabinoids act at the cellular molecular and epigenomic levels in myriad ways so their fallout could be very profound indeed. Indeed the most accurate paper in the whole area was the attached paper from Hawaii which identified 21 defects related to cannabis (see Forrester 2007, attached).

This paper by Forrester was also the only one to correctly explain the recent outbreak of babies born with no arms and cows born without legs in France near the Swiss border, but not in nearby Switzerland: cannabis is allowed in the food supply in France but not in Switzerland. So it seems obvious that cannabis is the cause there, just as was found earlier in Hawaii.

Closer to home, there was an outbreak of gastroschisis in 2011 it the northern rivers high cannabis area of NSW. This accords well with what is known from the literature as all seven papers examining cannabis and gastroschisis have reported a positive relationship. However the relationship was covered up at that time due to grossly incompetent statistics (the Bonferroni multiple testing correction which was erroneously applied was 16 times excessive to what it should have been; 150 vs. 9), a statistical analysis which I am told was done in Sydney. i.e. Major public heath blunders for which Queensland tax payers have foot the bill for many years!

Were you aware that the costs of care for cannabis related defects falls on the Queensland taxpayer at over five times the rates of non-cannabis related defects??? Obviously most of this is coming from the neighbouring high cannabis growing and consuming areas in northern NSW. This data is show up in Queensland health's own data summarized graphically below.

My recent submissions to FDA along these lines are also attached.

Should you wish to learn more I am of course happy to provide further information should you require it at that time.

Indeed I would call upon you not to be swayed by the folly of public opinion and informed media mass hysteria, but I would call upon you to work with serious practising clinicians and public health personnel to broadcast far and wide the now increasingly obvious harms of cannabis and to dispel the well-known, radically false view of cannabis as a somehow "soft drug."

I was also particularly concerned that my colleagues in Colorado are seeing an epidemic of obviously damaged and abnormal children there, just as I am here amongst my drug addicted patients and their offspring.

The public health implications could hardly be more obvious – or more dramatic. We are thus not in the least surprised to see these trends which we observe every day in our clinics, showing up in national epidemiology figures.

Similar concerns apply to mental health in young people. Again major US surveys show a close concordance, both over time and across geographical space, between cannabis consumption and severe and abnormal mental health outcomes. Again I am involved with senior statistical and data science analysts to report these trends further in the

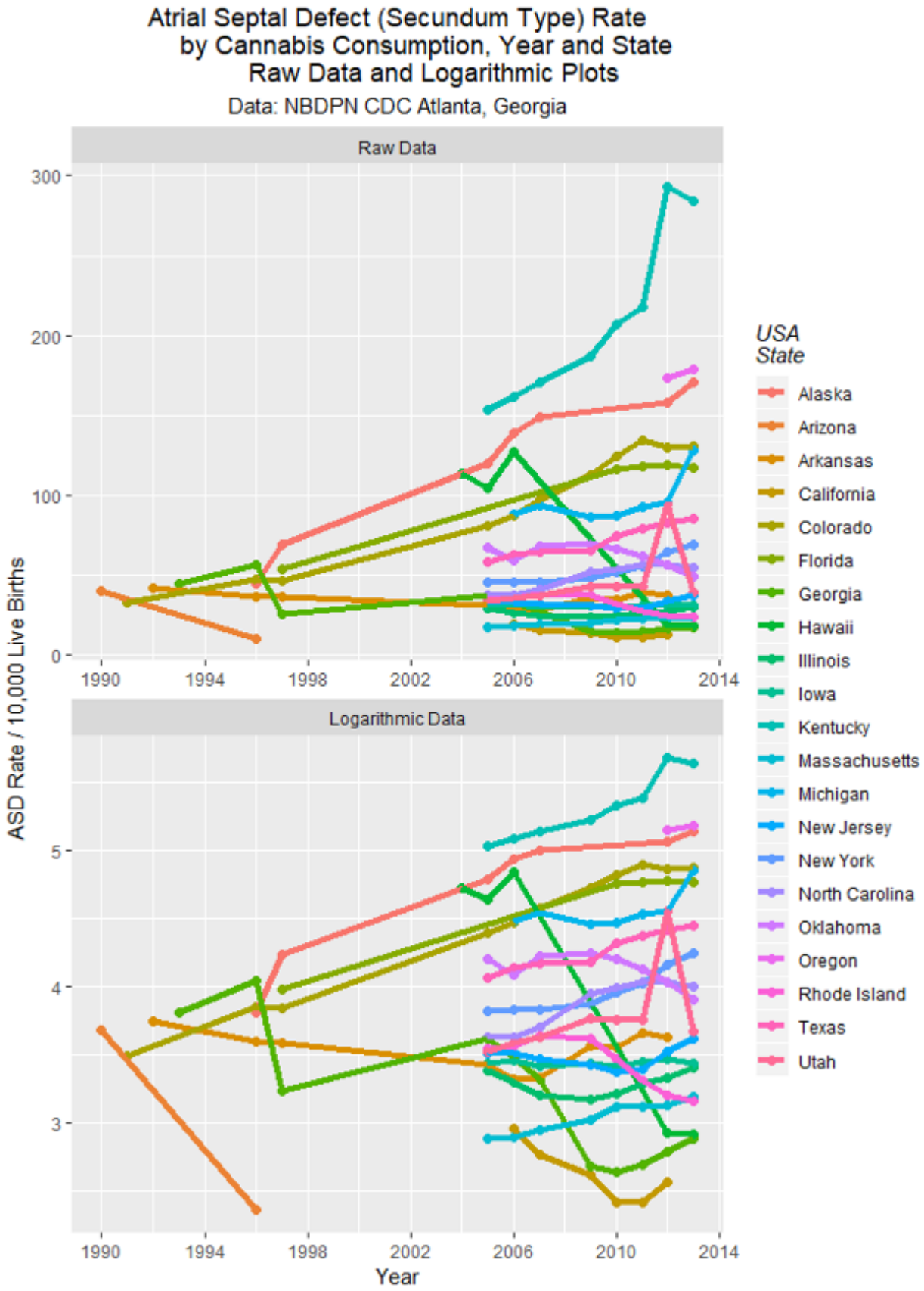
research literature. This close link however is well recognized by the Substance Abuse and Mental Health Administration (SAMHSA) in USA.

For information you will be interested to learn that I am now a full Professor of Medicine at both the Edith Cowan University and University of Western Australia in Perth.

You will also be aware that I have had one of the largest practices in addiction medicine in Queensland of any doctor for the last 20 years. This is a field in which I have extensive practical and academic involvement.

Yours sincerely,

Professor Dr. Stuart Reece,
[redacted]
Brisbane,
Queensland.


Atrial Septal Defect (Secundum Type) Rate
by Cannabis Consumption, Year and State
Raw Data and Logarithmic Plots
Data: NBDPN CDC Atlanta, Georgia
Raw Data
Logarithmic Data
ASD Rate / 10,000 Live Births
Year
USA
State
Alaska
Arizona
Arkansas
California
Colorado
Florida
Georgia
Hawaii
Illinois
Iowa
Kentucky
Massachusetts
Michigan
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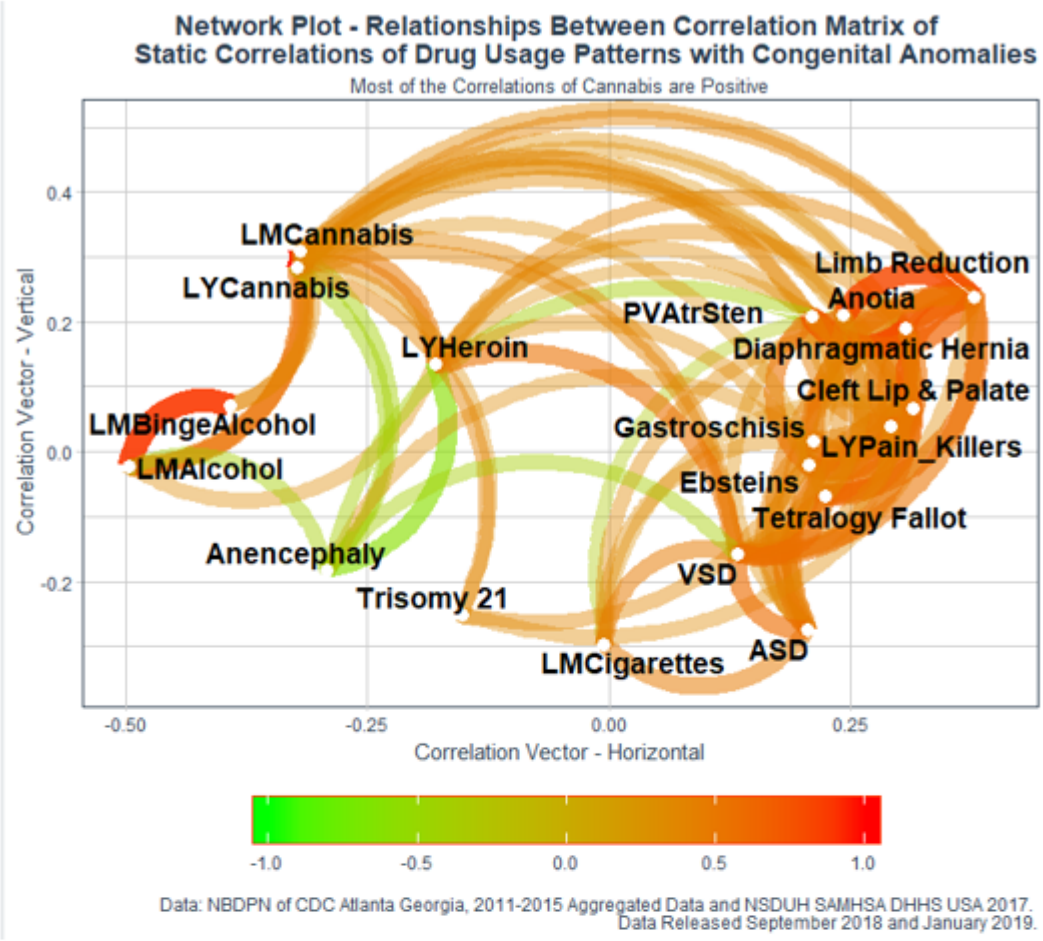
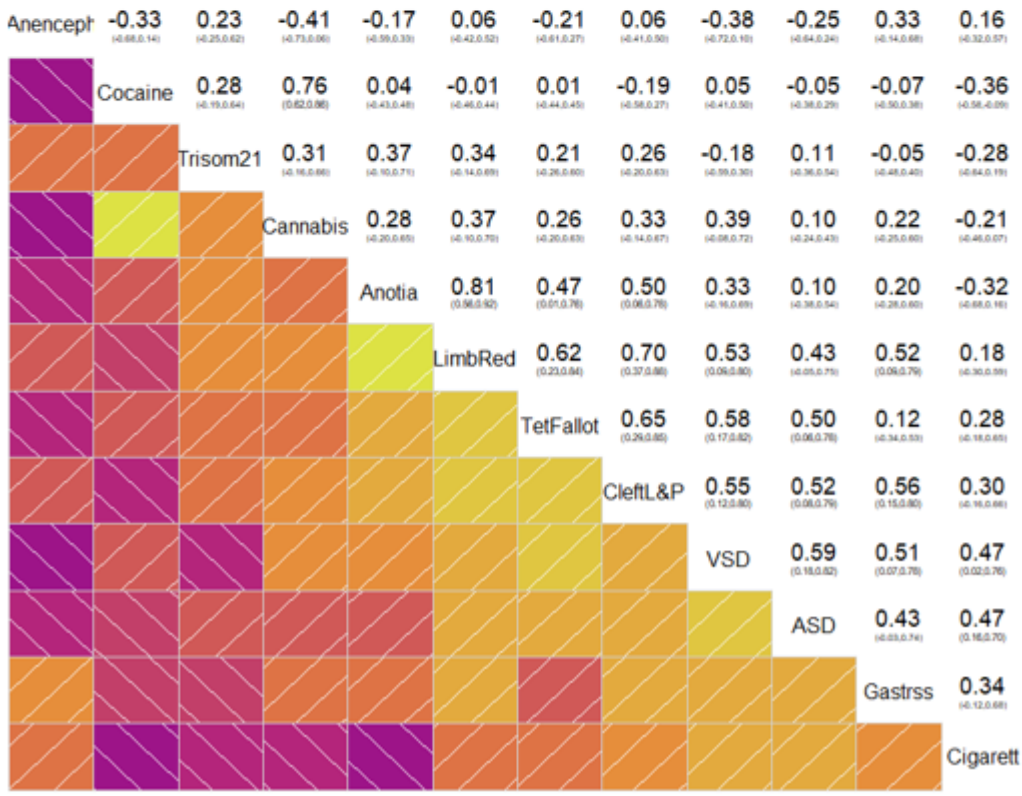
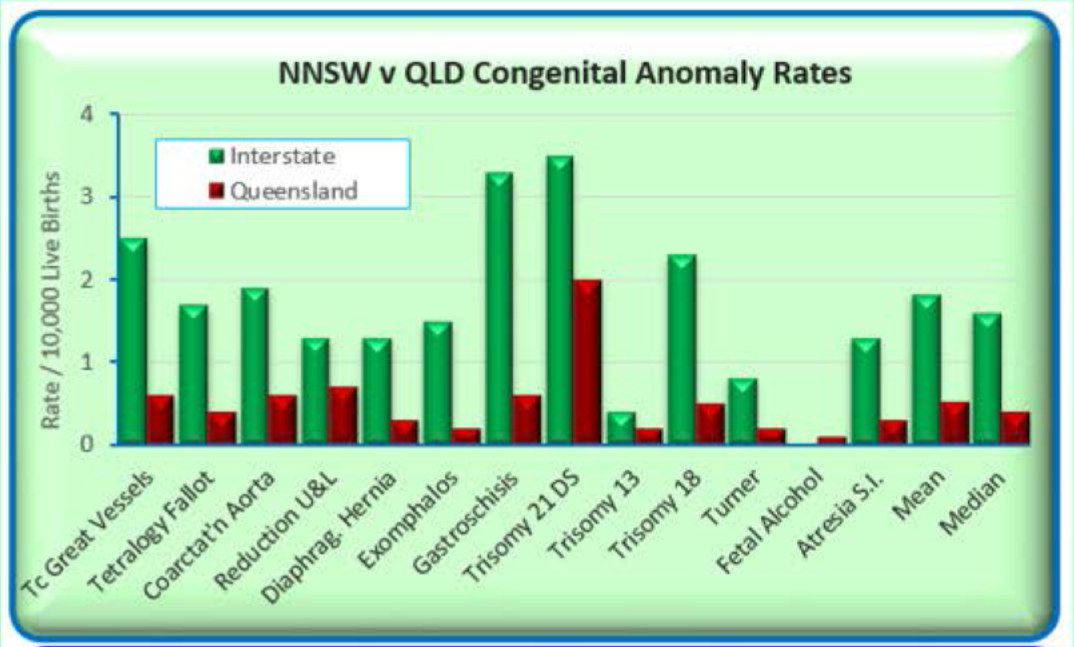
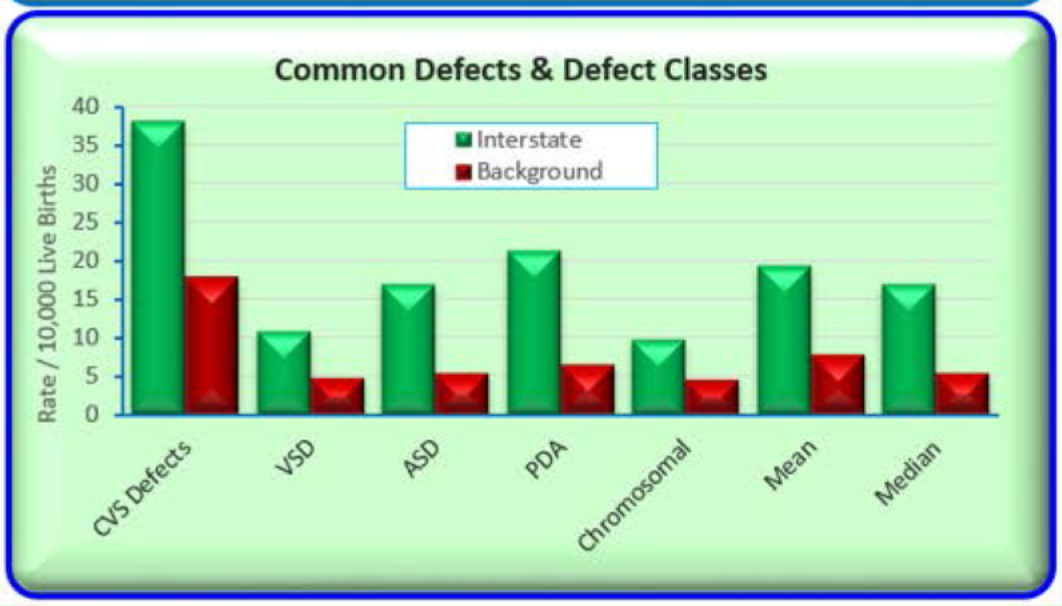


Corrgram - Relationships of Various Birth Defects to Selected Drug Exposure



QLD v NNSW Major and Minor Congenital Anomaly Rates





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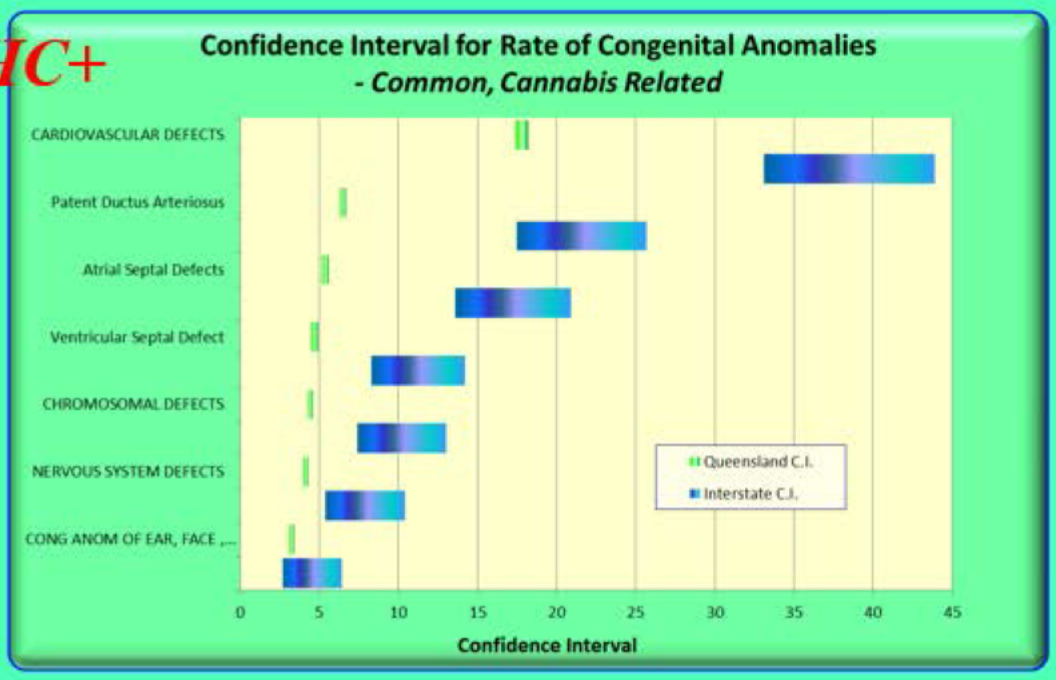
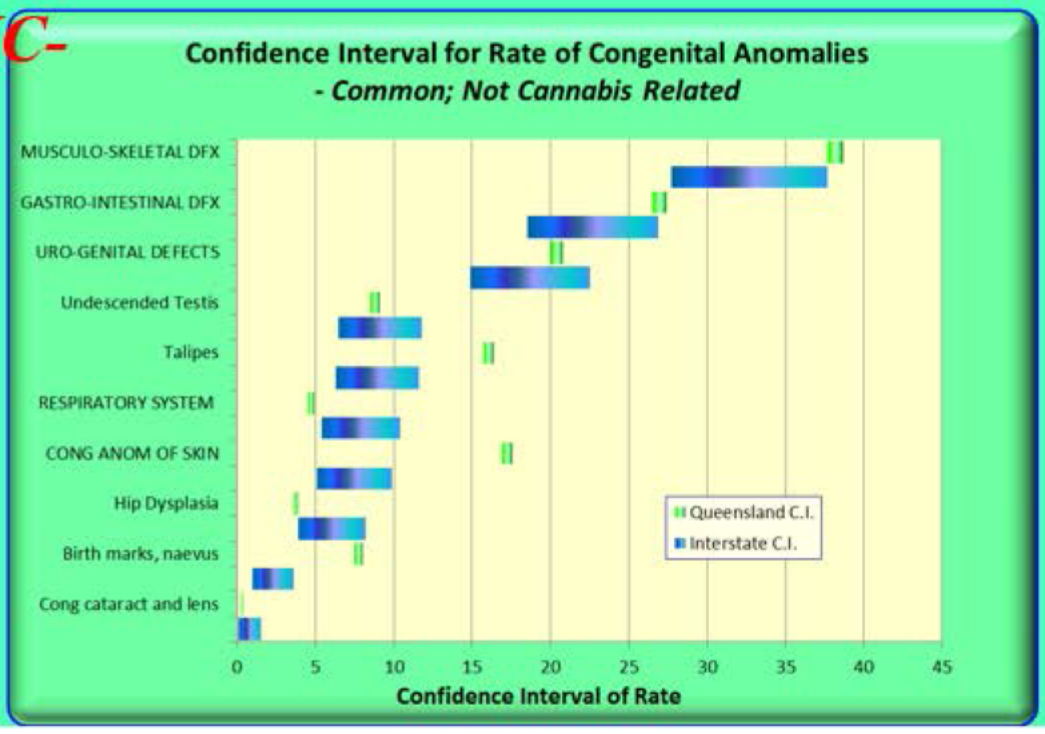
All Anomalies – C.I.'s of Rates - Common, THC+ v THC-

THC+



THC-

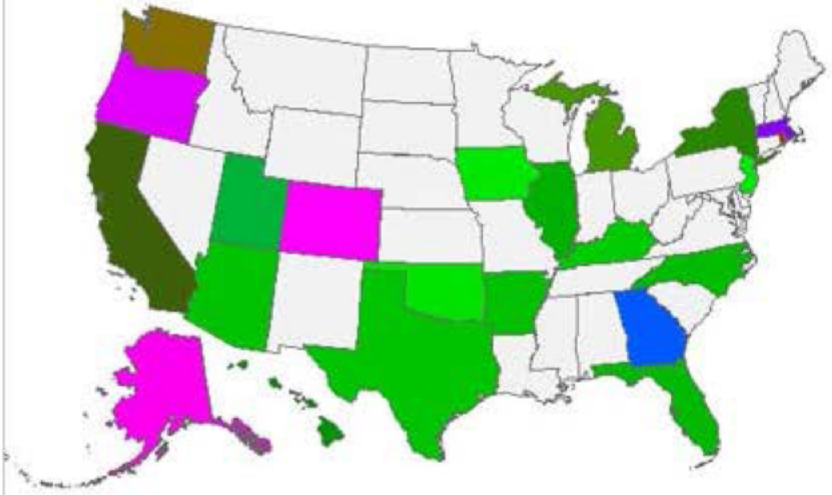


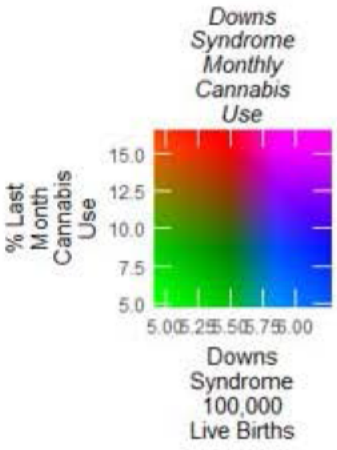
Chromsomal Trisomies, 2010-

Trisomy 21 – Downs Syndrome

Downs Syndrome Rate by Cannabis Use by USA State

NBDPN of CDC Atlanta Georgia, 2010-2014 Aggregated Data
and NSDUH SAMHSA DHHS USA 2014-2016





THE UNIVERSITY OF WESTERN AUSTRALIA

Associate Professor Dr. Stuart Reece
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April 16th 2018

Dockets Management Staff (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Rm. 1061
Rockville, MD 20852

Re: Department of Health and Human Services, Food and Drug Administration [Docket No. FDA-2018-N-1072]: International Drug Scheduling; Convention on Psychotropic Substances; Single Convention on Narcotic Drugs; Cannabis Plant and Resin; Extracts and Tinctures of Cannabis; Delta-9-Tetrahydrocannabinol; Stereoisomers of Tetrahydrocannabinol; Cannabidiol; **Request for Comments** (FR Doc. 2018-07225).

Re: ***Re-Scheduling of Cannabinoids in USA - Tetrahydrocannabinol and Cannabidiol Related Arteriopathy, Genotoxicity and Teratogenesis***

I am very concerned about the potential for increased cannabis availability in USA implied by full drug legalization; however, a comprehensive and authoritative submission of the evidence would take weeks and months to prepare. Knowing what we know now and indeed, what has been available in the scientific literature for a growing number of years concerning a myriad of harmful effects of marijuana, marijuana containing THC should not be reclassified. These effects that are now well documented in the scientific literature include, alarmingly, harm involving reproductive function and birth anomalies as a result of exposure to or use of marijuana with THC.

In addition to all of the usual concerns which you will have heard from many sources including the following I have further particular concerns:

1) Effect on developing brains [1-15]
2) Effect on driving [16-26]
3) Effect as a Gateway drug to other drug use including the opioid epidemic [27-30]
4) Effect on developmental trajectory and failure to attain normal adult goals (stable relationship, work, education) [17,31-43]
5) Effect on IQ and IQ regression [13,44-48]
6) Effect to increase numerous psychiatric and psychological disorders [49-62]
7) Effect on respiratory system [63-85]
8) Effect on reproductive system [7,86-91]
9) Effect in relation to immunity and immunosuppression [92-108]
10) Effect of now very concentrated forms of cannabis, THC and CBD which are widely available [109,110]
11) Outdated epidemiological studies which apply only to the era before cannabis became so potent and so concentrated [110].

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These issues are all well covered by a rich recent literature including reviews from such major international authorities as Dr Nora Volkow Director of NIDA at NIH [1,3,5,110-112], Professor Wayne Hall [113-117] and others [118].

Cannabinoid Therapeutics

In my view the therapeutic effects of cannabinoids have been wildly inflated by the press.

Moreover, with over 1,000 studies listed for cannabinoids on clinicaltrials.gov, the chance of a type I experimental error, or studies being falsely reported to be positive when in fact they are not, is at last 25/1,000 at the 0.05 level.

THC as dronabinol is actually a failed drug from USA which has such a high incidence of side effects that it was rarely used as superior agents are readily available for virtually all of its touted and alleged therapeutic applications. My American liaisons advise that dronabinol sales have climbed in recent times as patients use it as a ruse to avoid detection of cannabinoid use at work in states where it is not yet legal. So when I call is a failed therapeutic I mean in a traditional sense, not in the novel way it is now applied for flagrantly flouting the law.

In considering the alleged benefits of cannabis one has to be particularly mindful of cannabis addiction in which cannabinoids will alleviate the effect of drug withdrawal as they do in any other addiction. Moreover, the fact that cannabis itself is known to cause both pain and nausea, greatly complicates the interpretation of many studies.

I also have the following concerns which relate in sum to the **arteriopathy and vasculopathy** and the **genotoxicity of cannabis, tetrahydrocannabinol and likely including *cannabidiol* and various other cannabinoids**:

Cannabinoid Arteriopathy

12) Cannabis is now known to have an important arteriopathic effect and cardiovascular toxic effect [5,110,119-183]. Particularly noteworthy amongst these various reports are two reports by Dr Nora Volkow in 2014, the Director of the National Institute of Drug Abuse at NIH to the New England Journal of Medicine which together document the adverse cardiovascular and cerebrovascular effects of cannabis at the epidemiological level [5,110]; a report from our own clinic in 2016 documenting the effect of cannabis to increase cardiovascular aging to BMJ Open [183]; a series of reports showing a fivefold increase in the rate of heart attack within one hour after cannabis smoking [121-123]; several reports of cannabis related arteritis [162,163,168,170,171]; other reports of the cerebrovascular actions of cannabis [184-187]; documentation that cannabis exposure increases arterial stiffness and cardiovascular and organismal aging [183]; and a recent report showing that human endothelial vascular function – vasodilation - is substantially inhibited within just *one minute* of cannabis exposure [188].

13) It is also relevant that a synthetic cannabinoid was recently shown to directly induce both thromboxane synthase and lipoxygenase, and so be directly vasoconstrictive, prothrombotic and proinflammatory [189].

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14) Vascular aging, including both macrovascular and microvascular aging is a major pathological feature not only because most adults in western nations die from

myocardial infarction or cerebrovascular accidents, but also because local blood flow and microvascular function is a key determinant of stem cell niche activity in many stem cell beds. This has given rise to the vascular theory of aging which has been produced by some of the leading researchers at the National Health Lung and Blood Institute at NIH, amongst many others [190-192]. It can thus be said not only that "You are as old as your (macrovascular) arteries", but also that "you are as old as your (microvascular) stem cells." Hence the now compelling evidence for the little known arteriopathic complications of cannabis and cannabinoids, carry very far reaching implications indeed. This was confirmed directly in the clinical study of arterial stiffness from my clinic mentioned above [119].

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15) Whilst aging, myocardial infarction and cerebrovascular accidents are all highly significant outcomes and major public health endpoints, these effects assume added significance in the context of congenital anomalies. Some congenital defects, such as gastroschisis, are thought to be due to a failure of vascular supply of part of the anterior abdominal wall [193-198]. Hence in one recent study the unadjusted odds ratio of having a gastroschisis pregnancy amongst cannabis users (O.R.=8.03, 95%C.I. 5.63-11.46) was almost as high as that for heroin, cocaine and amphetamine users (O.R.= 9.35, 95%C.I. 6.64-13.15), and the adjusted odds ratio for any illicit drug use (of which was 84% cannabis) was O.R.=3.54 (95%C.I. 2.22-5.63) [199] and for cannabis alone was said by these Canadian authors to be O.R.=3.0 [200]. Hence cannabis related vasculopathy - arteriopathy beyond its very serious implications in adults also carries implications for paediatric and congenital disorders and may also *constitute a major teratogenic mechanism.*

Cannabinoid Genotoxicity and Teratogenesis

16) Cannabis is associated with 11 cancers (lung, throat, bladder, airways, testes, prostate, cervix, larynx) including [201,202];

17) Four congenital and thus inherited cancers (rhabdomyosarcoma, neuroblastoma, ALL, AML and AMML) [201,202];

18) Sativex product insert in many nations carries standard warning against its use by males or females who might be having a baby [203].

19) Cannabis – and likely also CBD – is known to be associated with epigenetic changes [30] some of which are believed to be inheritable for at least four generations [204];

20) Cannabis is known to interfere with tubulin synthesis [205-209] and binding and it also acts via Stathmin so that microtubule function is impeded [210]. This leads directly to micronucleus formation [113,211,212]. Cannabis has been known to test positive in the micronucleus assay for over fifty years [113,117,211]. This is a major and standard test for genotoxicity. Micronucleus formation is known to lead directly to major chromosomal toxicity including chromosomal shattering – so-called chromothripsis – and is known to be associated with cell death, cancerogenesis and major foetal abnormalities [202,213-215].

21) Cannabis has also been linked definitively with congenital heart disease is a statement by the American Heart Association and the American Academy of Pediatrics in 2007 [216], on the basis of just three epidemiological studies, all done in the days before cannabis became so concentrated. Congenital heart defects have also been linked with the father's cannabis use [217] . Indeed, one study showed that paternal cannabis use was the strongest risk factor of all for preventable congenital cardiac defects [218].

22) Cannabis has also been linked with gastroschisis in at least ***seven cohort and case control studies*** [199,219-224] some of which are summarized in a Canadian Government Report [200]. In that report the geographic incidence of most major congenital anomalies closely paralleled the use of cannabis as described in other major Canadian reports [225]. The overall adjusted odds ratio for cannabis induction of gastroschisis was quoted by these authors as 3.0 [200].

23) Moreover, outbreaks of both congenital heart disease [226] and gastroschisis in North Carolina also paralleled the local use of cannabis in that state as described by Department of Justice Reports [227]. The incidence of gastroschisis was noted to double in North Carolina 1999-2001 in the same period the cannabis trade there was rising [228]. Figures of cannabis use in pregnant women in California by age were also recently reported to JAMA [229], age group trend lines by age group which closely approximate those reported by CDC for the age incidence of gastroschisis in the USA [230] (Figure 1). Importantly much of the cannabis coming into both North Carolina and Florida is said to originate in Mexico [227,231]. An eight-fold rise in the rate of gastroschisis has been reported from Mexico [232]. Gastroschisis has also risen in Washington state [233].

24) Cannabis has also been associated with 17 other major congenital defects by major Hawaiian epidemiological study reported by Forrester in 2007 when it was used alone [221]. When considered in association with other drug use – which in many cases cannabis leads to – cannabis use was associated with a further 19 major congenital defects.

25) In addition to the effect of cannabinoids on the epigenome and microtubules, cannabinoids have been firmly linked to a reduction of the ability of the cell to produce energy from their mitochondria [78,82,91,234-249]. An extensive and robust evidence base [244] now links cellular energy generation to the maintenance and care of cellular DNA [250-253]. Moreover, as the cellular energy charge falls so too DNA maintenance collapses, and indeed, the cell can spiral where its remaining energy resources, particularly as NAD+, are routed into failing and futile DNA repair, the cell slips into pseudohypoxic metabolism like the Warburg effect well known in cancerogenesis [254], NAD+ falls below the level required for further energy generation and cellular metabolism collapses. Hence this well-established collapse of the mitochondrial energy charge and transmembrane potential forms a potent engine of continuing and accelerating genotoxicity [255].

26) Moreover, the well documented decline in mitochondrial respiration induced by cannabinoids, including tetrahydrocannabinol, cannabidiol and anandamide [78,82,91,234-242,244-248] achieves particular significance in the light of the robustly documented decline in cellular energetics including NAD+ which not only occurs with age [251,256-268] but indeed, ***has now been shown to be one of the primary drivers of cellular and whole organismal aging*** [250-254,263,269-289]. This close parallel is illustrated in Figure 2. It follows therefore that *cannabinoid administration (including THC and CBD)* ***necessarily phenocopies cellular aging***. This implies of course that cannabinoid dependent patients are old at the cellular level. Indeed, normal human aging is phenocopied in the clinical syndrome of cannabinoid dependence which includes (most references are provided above):

1) Neurological deficits in:
    i) attention,
    ii) learning and
    iii) memory;
    iv) social withdrawal and disengagement and
    v) academic and
    vi) occupational underachievement
2) Psychiatric disorders including
    i) Anxiety,
    ii) Depression,
    iii) Mixed Psychosis
    iv) Bipolar Affective disorder and
    v) Schizophrenia,
3) Respiratory disorders including:
    i) Asthma
    ii) Chronic Bronchitis (increased sputum production)
    iii) Emphysema (Increased residual volume)
    iv) Probably increased carcinomas of the aerodigestive tract
4) Immune suppression which generally implies
    i) segmental immunostimulation in some parts of the immune system since
    the innate and adaptive immune systems exert profound homeostatic mechanisms in response to suppression of one of its parts;
    A Substantial literature on immunostimulation
5) Reproductive effects generally characterized by reduced
    i) Male and
    ii) Female fertility
6) Cardiovascular toxicity with elevated rates of
    i) Myocardial infarction
    ii) Cerebrovascular accident
    iii) Arteritis
    iv) Vascular age – vascular stiffness [119]
7) Genotoxicity in
    i) Respiratory epithelium and
    ii) Gonadal tissues.
8) Osteoporosis [290-300]

9) Cancers of the
    i) Head and neck
    ii) Larynx
    iii) Lung
    iv) Leukaemia
    v) Prostate
    vi) Cervix
    vii) Testes
    viii) Bladder
    ix) Childhood neuroblastoma
    x) Childhood acute lymphoblastic leukaemia
    xi) Childhood Acuter Myeloid and myelomonocytic leukaemia
    xii) Childhood rhabdomyosarcoma [201,202].

The issue here of course is that cannabinoid dependence therefore copies *without exception* all of the major disorders of old age, each of which is also faithfully phenocopied by cannabis dependence.

The most prominent disorders of older age include:

1) **Alzheimer's disease**
2) **Cardiovascular and cerebrovascular disease**
3) **Osteoporosis**
4) **Systemic inflammatory syndrome**
5) **Changes in lung volume and the mechanics of breathing**
6) **Cancers**

Hence this provides one powerful pathway by which cannabinoid exposure can replicate and phenocopy the disorders of old age.

This is not of course to suggest that this is the only such pathway. Obviously changes of the general level of immune activity, or alterations of the level of DNA repair occurring directly or indirectly associated with cannabis use can form similar such pathways: both are well documented in cannabis use and also in the aging literature as major pathways implicated in systemic aging. Nevertheless, the decline in mitochondrial energetics together with its inherent genotoxic implications does seem to be a particularly well substantiated and robustly demonstrated pathway which must give serious pause to cannabinoid advocates if the sustainability of the health and welfare systems is to be factored in together with any consideration of individual patient, advocate and industrial-complex rights.

27) The genotoxicity of THC, CBD and CBN has been noted against sperm since at least 1999 (Zimmerman and Zimmerman in Nahas "Marijuana and Medicine" 1999, Springer). This is clearly highly significant as sperm go directly into the formation of the zygote and the new human individual.

28) CB1R receptors are known to exist intracellularly on both the membranes of endoplasmic reticulum and mitochondria. In both locations they can induce organellar stress and major cell toxicity including disruption of DNA maintenance. Interestingly mitochondrial outer membrane CB1R's signal via a complex signaling chain involving the G-protein transduction machinery, protein kinase A and cyclic-AMP across the intermembrane space to the inner membrane and cristae, in a fashion replicating much of the G-protein signaling occurring at the cell membrane. This machinery is also implicated in mitonuclear signaling, and the mitonuclear DNA balance between mitochondrial DNA and nuclear DNA transcriptional control, which has long been implicated in inducing the mitochondrial unfolded protein cellular stress response cell aging, stem cell behaviour and DNA genotoxic mechanisms [248,301].

29) You are no doubt aware that human sperm are structured like express outboard motors behind DNA packets with layers of mitochondria densely coiled around the rotating flagellum which powers their progress in the female reproductive tract (Figure 3). These mitochondria also carry CB1R's and are significantly inhibited even at 100 nanomolar THC. The acrosome reaction is also inhibited [239].

30) A similar arrangement is shown in Figure 4, where mitochondria are shown in green surrounding the mitotic spindle (pink, with the chromosomes shown in blue), which is the cellular machinery and apparatus of cell division. Mitosis and meiosis, the classical processes of cell division, are highly energy dependent and mitochondria are clearly positioned strategically to supply the required energy for this process, just as they are positioned in proximity to the root of the sperm flagellum rotor in that situation.

31) Cannabidiol is known to act via the PPARγ system [101,302-308]. PPARγ is known to have a major effect on gene expression, reproductive and embryonic and zygote function during development [309-332] so that significant genotoxic and / or teratogenic effects seem inevitable via this route. Drugs which act in this class, known as the thiazolidinediones, are classed as category B3 in pregnancy and caution is indicated in their use in pregnancy and lactation.

32) The Report of the Reproductive and Cancer Hazard Assessment Branch of the Office of Environmental Health Hazard Assessment of the Health Department of California was mentioned above in connection with the carcinogenicity of marijuana smoke [333]. ***Since virtually all mutagens are also teratogens*** it follows therefore from the basic tenets of mutagenesis that if cannabis is unsafe as a known carcinogen it must also be at the very least a putative teratogen.

33) CBD has also been noted to be a genotoxic in other studies [334-336].

34) All of which points to major teratogenic activity for both THC and CBD.

Some of the quotations from Professor James Graham's classical book on the effects of THC in hamsters and white rabbits, the best animal models for human genotoxicity, bear repeating [337]:

a) "The concentration of THC was relatively low and the **malignancy severe**."
b) "40-100µg resin/ml there occurred **marked inhibition of cell division.**
c) "large total dose, Hamsters, 25-300mg/kg ..."oedema, **phocomelia**, **omphalocoele**, **spina bifida, exencephaly**, multiple malformations and **myelocoele**. **This is a formidable list**."
d) "It is to this **anti-mitotic action** that the authors attribute the **embryotoxic** action of cannabis."
e) "By such criteria resin or extract of cannabis would be **forbidden to women** during the first three months of **pregnancy**." [337]

Indeed, even from the other side of the world I have heard many exceedingly adverse reports from US states in which cannabis has been legalized including Colorado, Washington, Oregon, Florida and California [231,233,338-342]. Taken together the above evidence suggests that these negative reports stem directly from the now known actions of cannabis and cannabinoids, and are by no means incidental epiphenomena somehow related to social constructs surrounding cannabis use or the product forms, dosages, or routes of administration involved [343].

Cannabis that contains increasingly high levels of THC is now widely available, particularly in the jurisdictions where the use of cannabis has been legalized. This means that another major genotoxin, akin to Thalidomide, is being unleashed on the USA and the world. This is clearly a very grave, and. indeed, an entirely preventable occurrence.

Dr Frances Kelsey of FDA is said to have the public servant based at FDA who saved American from the thalidomide scandal which devastated so many other English-speaking nations including my own [344]. This occurred because the genotoxicity section of the file application with FDA was blank. It was blank because thalidomide tested positive in various white rabbit and guinea pig assays. ***It is these same tests which cannabis is known to have failed*** [88,337,345,346]. Dr Kelsey's photograph has been published in the medical press with President Kennedy for her service to the nation (Figure 5) [344]. The challenge to FDA at this time seems whether Science can triumph over agenda driven populism, its primary vehicle, the mass media, and its primary proximate driver the burgeoning cannabis industry. Since FDA is the Federal agency par excellence where Health Science is weighed, commissioned and thoughtfully considered the challenge in our time would appear to be no less.

Evidence to date does not suggest that major congenital malformations are as common after prenatal cannabis exposure as they are after prenatal thalidomide exposure. Nevertheless the qualitative similarities remain and indeed are prominent. It is yet to be seen whether the rate of congenital anomalies after cannabis are quantitatively as common: epidemiological studies in a high potency era have not been undertaken; and even the birth defects rates from most birth defects registers in western nations including that held by CDC, Atlanta appear to be seriously out of date at the time of writing. Moreover the non-linear dose response curve in

many cannabis genotoxicity studies which includes a sharp knee bend upwards beyond a certain threshold level which suggests that we could well be in for a very unpleasant quantitative surprise. At the time of writing this remains to be formally determined.

Dr Bertha Madras, Professor of Addiction Psychiatry at Harvard Medical School has recently argued against re-scheduling of cannabis. Her comments include the following:

> *"Why do nations schedule drugs? ..... Nations schedule psychoactive drugs because we revere this three-pound organ (of our brain) differently than any other part of our body. It is the repository of our humanity. It is the place that enables us to write poetry and to do theater, to conjure up calculus and send rockets to Pluto three billion miles away, and to create I Phones and 3 D computer printing. And that is the magnificence of the human brain. Drugs can influence (the brain) adversely. So, this is not a war on drugs. This is a defense of our brains, the ultimate source of our humanity"* [347].

I look forward to seeing the comments that you post concerning the reasons why the classification for marijuana should not be changed and that, indeed, the public should be alerted to the very harmful effects of marijuana with THC, especially in light of the wide range of marijuana's harmful effects and the high potency of THC in today's marijuana and in light of the idiosyncratic effects of marijuana of even low doses of THC and owing to the certain risk of harm to progeny and babies born to users of marijuana.

Please feel free to call on me if you would like further information concerning the research to which I have referred herein.

Yours sincerely,

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Figure Captions

Figure 1: Cannabis Use Rates in Pregnancy in California and Gastroschisis Rates USA, by Age Groups

Figure 2: Close Parallel between the Collapse of Mitochondrial NAD+ Dependent Respiration in Complexes I, II and IV and the Decline of NAD+ with Physiological Aging.

Figure 3: Sperm swimming demonstrating how mitochondria are wrapped around the central axel of the flagellum to provide local energy where it is needed.

Figure 4: Mitochondria (green) surrounding the mitotic spindle (made of microtubuiles shown in pink) which carry the chromsomes (blue) at the time of cell division. Photo taken from NIH laboratories (http://https//visualsonline.cancer.gov/details.cfm?imageid=10708).

Figure 5: Presentation of Dr Frances Kelsey of FDA to President John F Kennedy.

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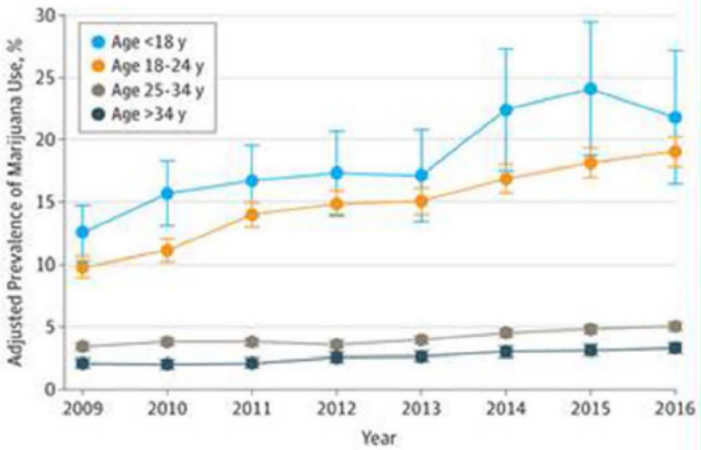
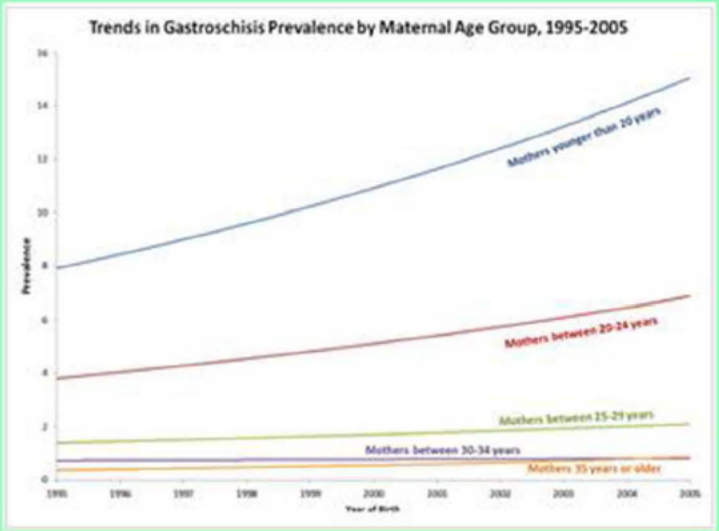
Cannabis Use in Pregnancy, California & Gastroschisis Rates, USA by Age Groups

Figure 2. View Large Download



Adjusted Prevalence of Marijuana Use Among 279 457 Pregnant Females in KPNC by Age, 2009-2016



December 26, 2017

Trends in Self-reported and Biochemically Tested Marijuana Use Among Pregnant Females in California From 2009-2016

Kelly C. Young-Wolff, PhD, MPH[1]; Lue-Yen Tucker, BA[1]; Stacey Alexeeff, PhD[2]; Mary Anne Armstrong, MA[1]; Amy Conway, MPH[2]; Constance Weisner, DrPH[3]; Nancy Golar, MD[4]

> Author Affiliations | Article Information

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http://www.cdc.gov/ncbddd/birthdefects/features/gastroschisis-key-findings.html

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Decline in NAD+ from Cannabinoids Mimics those Occurring with Age

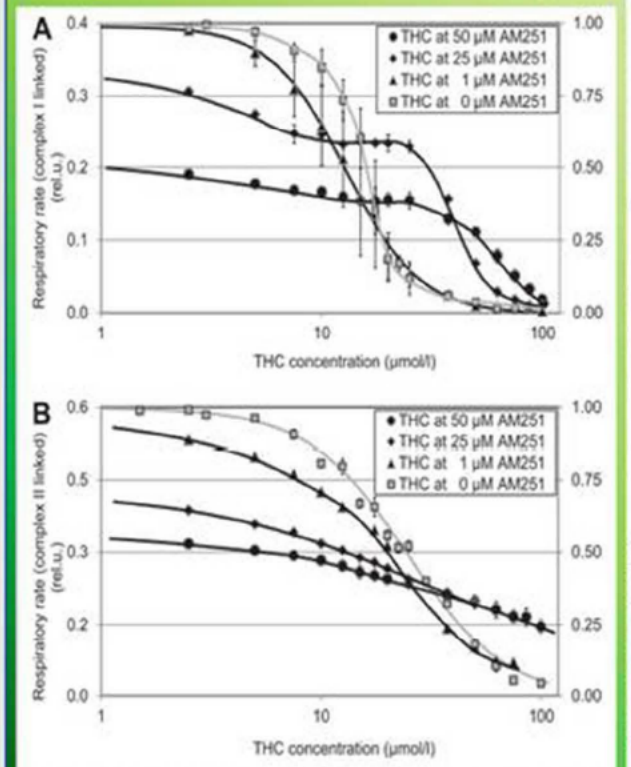


Fig. 3. THC-induced inhibition of the respiratory rate in pig brain mitochondria incubated in substrates of electron supply through (**A**) complex I or (**B**) complex II.

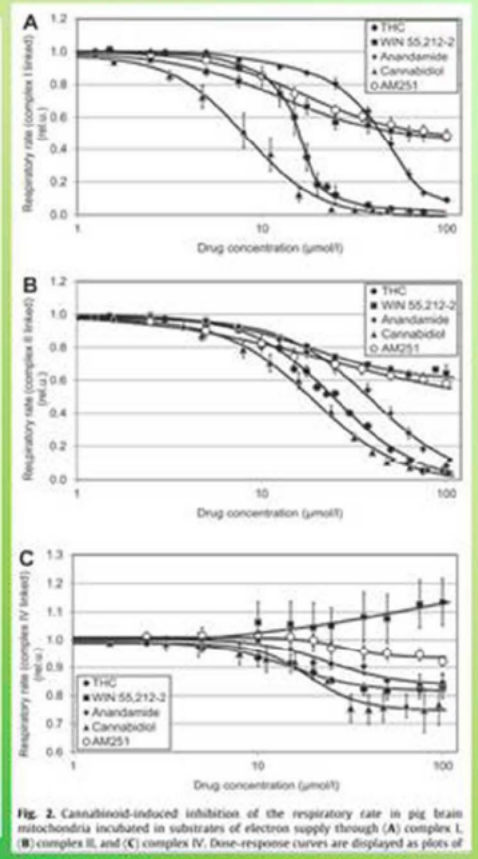
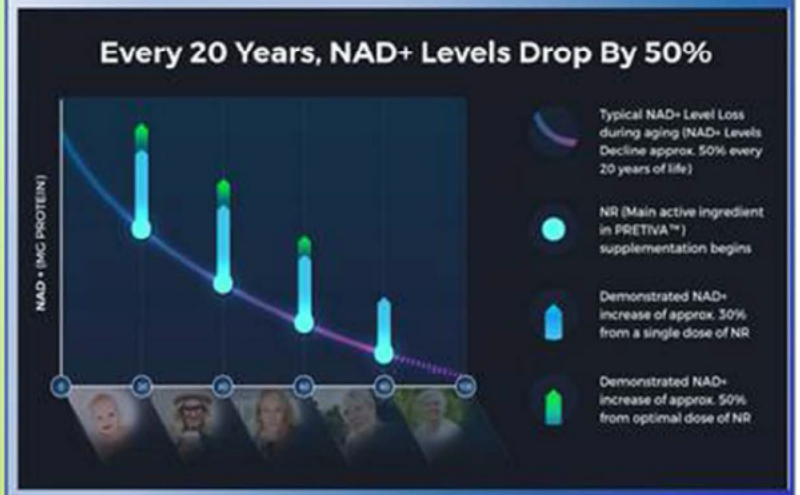


Fig. 2. Cannabinoid-induced inhibition of the respiratory rate in pig brain mitochondria incubated in substrates of electron supply through (**A**) complex I, (**B**) complex II, and (**C**) complex IV. Dose-response curves are displayed as plots of

Toxicol Lett. 2014 Nov 18;231(1):62-71. doi: 10.1016/j.toxlet.2014.09.002. Epub 2014 Sep 6.

Cannabinoid-induced changes in respiration of brain mitochondria.

Fišar Z[1], Singh N[2], Hroudová J[3].



http://dailytrendsetter.com/health/pretiva-a-pill-to-slow-the-aging-process-would-you-take-it/

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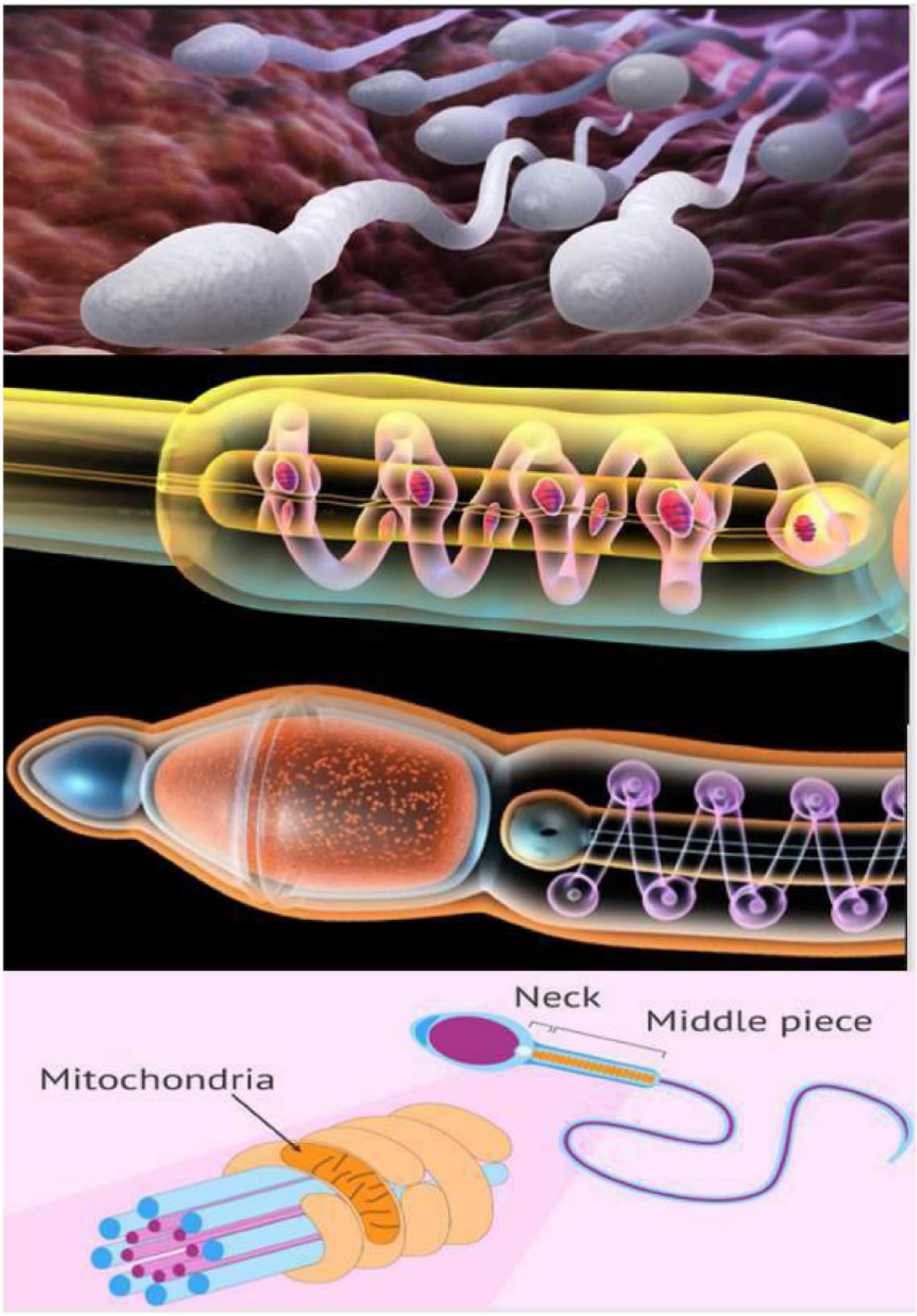

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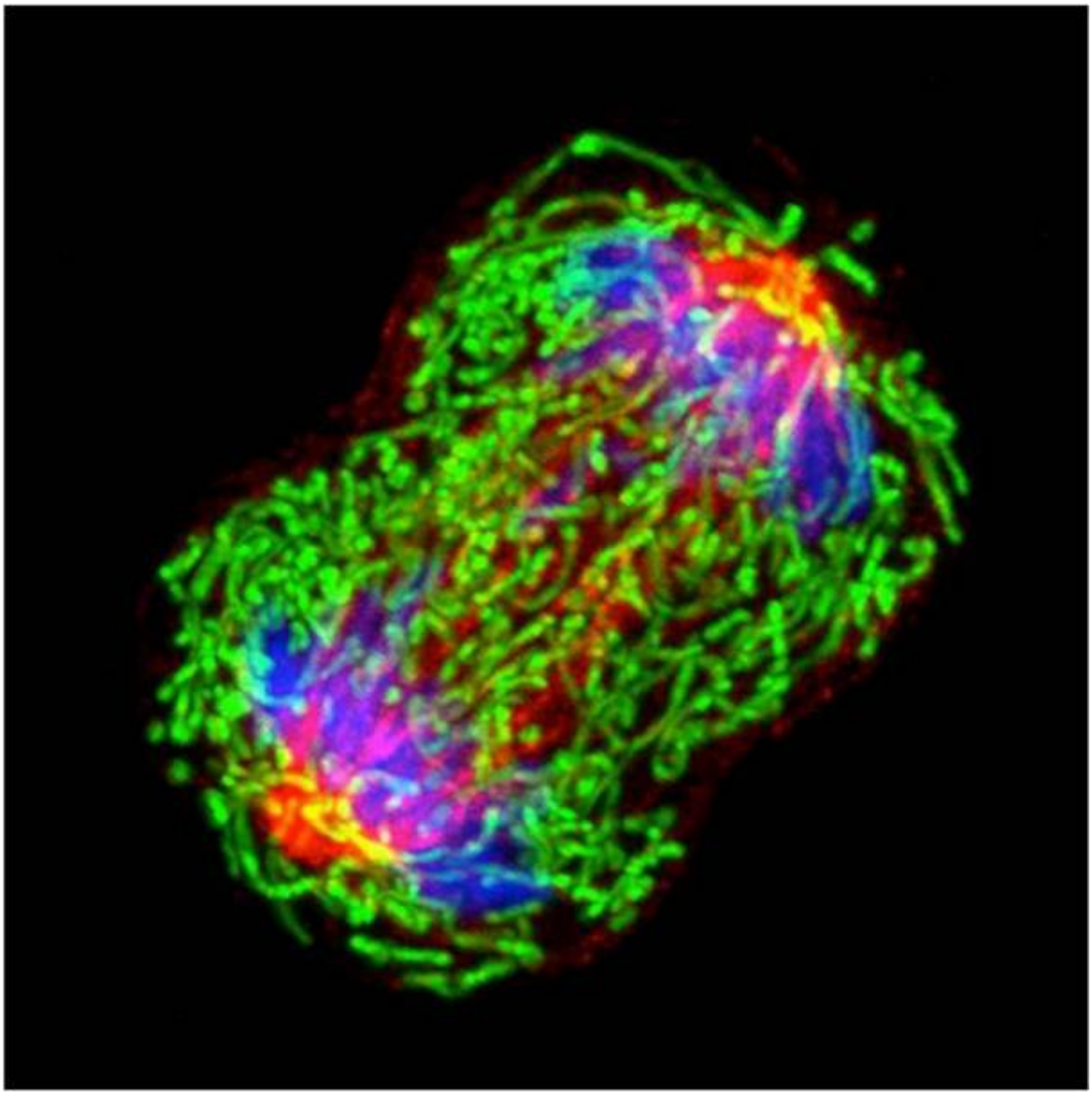
Cell Dividing



http://https//visualsonline.cancer.gov/details.cfm?imageid=10708

Dr Frances Kelsey @ FDA
Saved USA from Thalidomide

Figure 2. Dr Frances Kelsey is awarded the President's Award for Distinguished Federal Civilian Service from President John F. Kennedy in 1962.

Therapeutic Advances in Hematology *Review*

The rise, fall and subsequent triumph of thalidomide: lessons learned in drug development

Waqas Rehman, Lisa M. Arfons and Hillard M. Lazarus

THE UNIVERSITY OF WESTERN AUSTRALIA

Associate Professor Dr. Stuart Reece
MBBS (Hons.), FRCS(Ed.), FRCS(Glas.), FRACGP, MD(UNSW).
School of Psychiatry and Clinical Neurosciences

22nd April 2018

Dockets Management Staff (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Rm. 1061
Rockville, MD 20852

Re: Department of Health and Human Services, Food and Drug Administration [Docket No. FDA-2018-N-1072]: International Drug Scheduling; Convention on Psychotropic Substances; Single Convention on Narcotic Drugs; Cannabis Plant and Resin; Extracts and Tinctures of Cannabis; Delta-9-Tetrahydrocannabinol; Stereoisomers of Tetrahydrocannabinol; Cannabidiol; **Request for Comments** (FR Doc. 2018-07225).

Re: ***Novel Considerations for the FDA Evaluation of Re-scheduling Cannabinoids in United States: Cannabinoid Genotoxicity and Structural and Neurobehavioral Teratogenicity***

There exists sufficient empirical data from cellular to epidemiological studies to warrant caution in the use cannabinoids including cannabidiol as recreational and therapeutic agents.

Cannabinoids bind to CB1R receptors on neuronal mitochondrial membranes [1-7] where they can directly disrupt key functions [8-12] including cellular energy generation, DNA maintenance and repair, memory and learning [1-7,9,10,13-24].

Empirical literature associates cannabinoid use with CB1R-mediated vasospastic and vasothrombotic strokes, myocardial infarcts, arrhythmias [25-98] and arteritis [25,77,78,99-106]. Cannabis has been associated with increased cardiovascular stiffness and vascular aging, a major surrogate for organismal aging [107]. In the pediatric-congenital context CB1R-mediated cannabis vasculopathy forms a major pathway to teratogenesis including VSD, ASD, endocardial cushion defects, several other cardiovascular anomalies [75,108] and, via the omphalo-vitelline arterial CB1R's [25], gastroschisis [108-114]. Cannabis has been linked with several other malformations including hydrocephaly [108]. Cannabinoids also induce epigenetic perturbations [115-123]; and, like thalidomide [124-126], interfere with tubulin polymerization [127-132] and the stability of the mitotic spindle precipitating micronucleus formation [129,133-142], chromosomal shattering (chromothripsis) [129,143-157] providing further major pathways to genotoxicity .

Assuming validity of the above data, increased levels of both adult and neonatal morbidity should accompany increased cannabis use. The "Colorado Responds to Children with Special Needs" (CRCSN) program tracked congenital anomalies 2000-2013 [158]. Importantly this data monitors the teratological history of Colorado since 2001 when the state was first advised that intrastate cannabis would not be prosecuted by the Federal Government. In 2012 medical cannabis was legalized and in 2014 cannabis was completely legalized.

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Over the period 2000-2013 Colorado almost doubled its already high congenital anomaly rate rising from 4,830 anomalies / 65,429 births (7.4%) to 8,165 / 65,004 (12.6%; Figure 1); the US mean is 3.1%. Major cardiovascular defects rose 61% (number and rate); microcephaly rose 96% (from 30 to 60 cases peaking at 72 in 2009); and chromosomal anomalies rose 28% (from 175 to 225, peaking at 264 in 2010; Figure 2-7). Over the whole period this totals to 87,772 major congenital anomalies from 949,317 live births (9.25%).

The use of cannabis in Colorado can be determined from the SAMHSA National Survey on Drug Use and Health. A close correlation is noted between major congenital anomaly rates and rates of cannabis use in Coloradans >12 years (R=0.8825; P=0.000029; Figure 8). Although data is not strictly comparable across U.S. registries, the Colorado registry is a passive rather than active case-finding registry and so might be expected to underestimate anomaly rates. Given the Colorado birth rate remained almost constant over the period 2000-2013, rising only 3.6%, a simple way to quantitate historical trends is to simply project forwards the historical anomaly rate and compare it to the rise in birth numbers. However rather than remaining relatively stable in line with population births, selected defects (left hand column Table 1) have risen several times more than the birth rate (right hand column).

Colorado had an average of 67,808 births over the period 2000-2013 and experienced a total of 87,772 birth defects, 20,152 more than would have been predicted using 2000 rates. Given the association between cannabis use and birth defects and the plausible biological mechanisms, cannabis may be a major factor contributing to birth congenital morbidity in Colorado. If we accept this and apply the "Colorado effect" to the over 3,945,875 births in USA in 2016 we calculate an excess of 83,762 major congenital anomalies annually nationwide if cannabis use rises in the US to the level that it was in Colorado in 2013.

In reality both cannabis use and cannabis concentration is rising across USA following legalization which further implies that the above calculations represent significant underestimations [159,160]. This CRCSN data series terminates in 2013 prior to full legalization in 2014. Moreover parents of children harbouring severe anomalies may frequently elect for termination, which will again underestimate numbers of abnormal live births.

In California 7% of all pregnant mothers were recently shown to test positive for cannabis exposure, including almost 25% of teenage mothers in 2015 so cannabinoids clearly constitute a significant population-wide teratological exposure [161]. This is particularly relevant to cannabis genotoxicity as many studies show a dramatic up-tick in genotoxic effect in the dose-response curve for both tetrahydrocannabinol and cannabidiol above a certain threshold dose as higher, sedating levels are reached [132,136,162-166]. Cannabis is usually used amongst humans for its sedative effects.

Other examples of high congenital anomaly rates accompanying increased cannabis use include North Carolina [167-169], Mexico [170-175], Northern Canada [111,176-178], New Zealand and the Nimbin area in Australia [179-182].

The above data leave open the distinct possibility that the rate of congenital anomalies from significant prenatal paternal or maternal cannabis exposure may become substantial.

With over 1,000 trials listed on clincaltrials.gov the chance of a type I experimental error for cannabinoid therapeutics and a falsely positive trial finding is at least 25/1,000 trials at the 5% level.

The major anomaly rate is just the "tip of the iceberg" of the often subtle neurobehavioral teratology of Foetal Cannabinoid Syndrome (FCS) following antenatal cannabinoid exposure characterized by attention, learning, behavioral and social deficits which in the longer term impose significant educational, other addiction and welfare costs - and is clearly more common [121,183-225]. Foetal Alcohol Syndrome (FAS) is known to be epigenetically mediated [226-251] and foetal alcohol is known to act via CB1R's [186,203,206-208,210,216,252-259]. Cannabis has significant and heritable epigenetic imprints in neural, immune and germ cell (sperm) tissues [20,117,119,120,122,260-262], and epigenomic disruption has been implicated in FCS [241]. CB1R-mediated disruption by disinhibition of the normal gamma and theta oscillatory rhythms of the forebrain which underpin thinking, learning and sanity have been implicated both in adult psychiatric disease and the neurodevelopmental aspects of FCS [211].

All of this implies that in addition to usually short-term therapy-oriented clinical trials, longer term studies and careful twenty-first century next generation studies will be required to carefully review inter-related genotoxic, teratologic, epigenetic, transcriptomic, metabolomic, epitranscriptomic and long term cardiovascular outcomes which appears to have been largely overlooked in extant studies – effects which would appear rather to have taken Coloradans by surprise. Congenital registry data also needs to be open and transparent which it presently is not. We note that cannabidiol is now solidly implicated in genotoxicity [134,263-269]. Governments are duty-bound to carefully weigh and balance the implications of their social policies; lest like Colorado, we too unwittingly create a "Children with Special Needs Program" [158].

These data also directly imply that young adults, as the very group which most consumes cannabis [160,161,270-273] is the very group which most requires protection from its reproductive, genotoxic and teratogenic effects.

Yours sincerely,

Assoc. Prof. Dr. Stuart Reece.
University of Western Australia and
Edith Cowan University,
Perth,
Australia.

Table 1.:

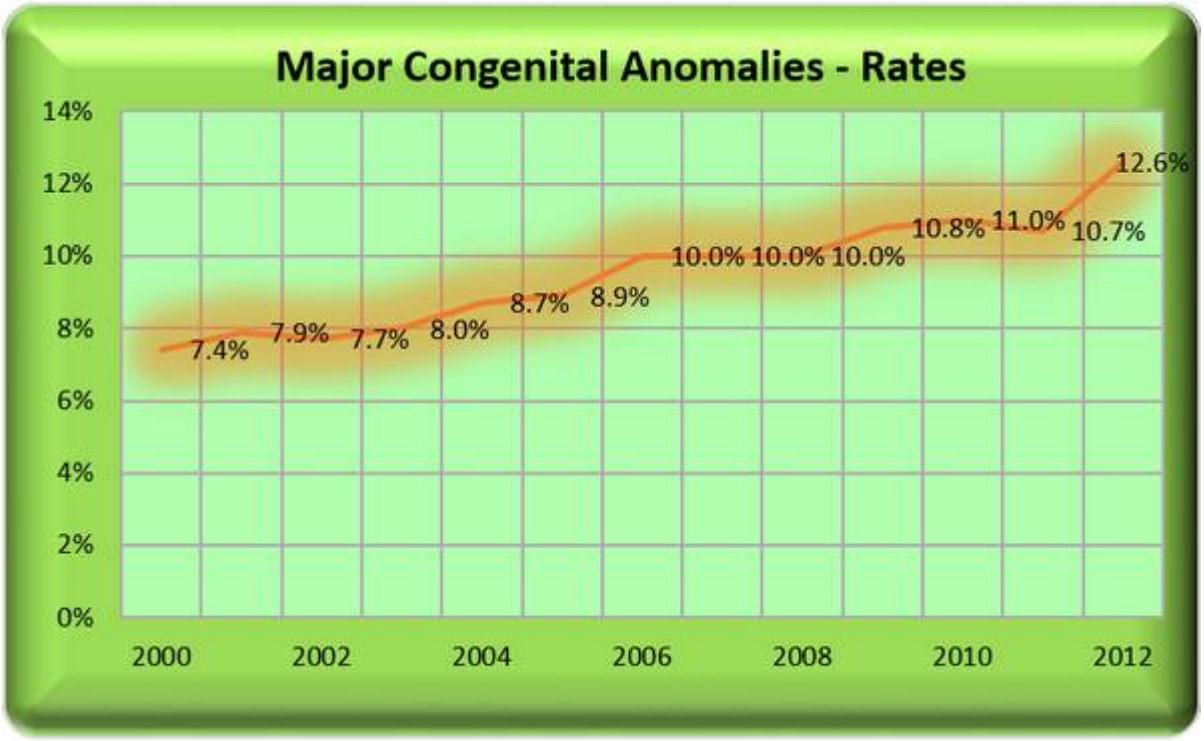
Cumulative Data for Colorado

Birth Defects 2000-2013 *

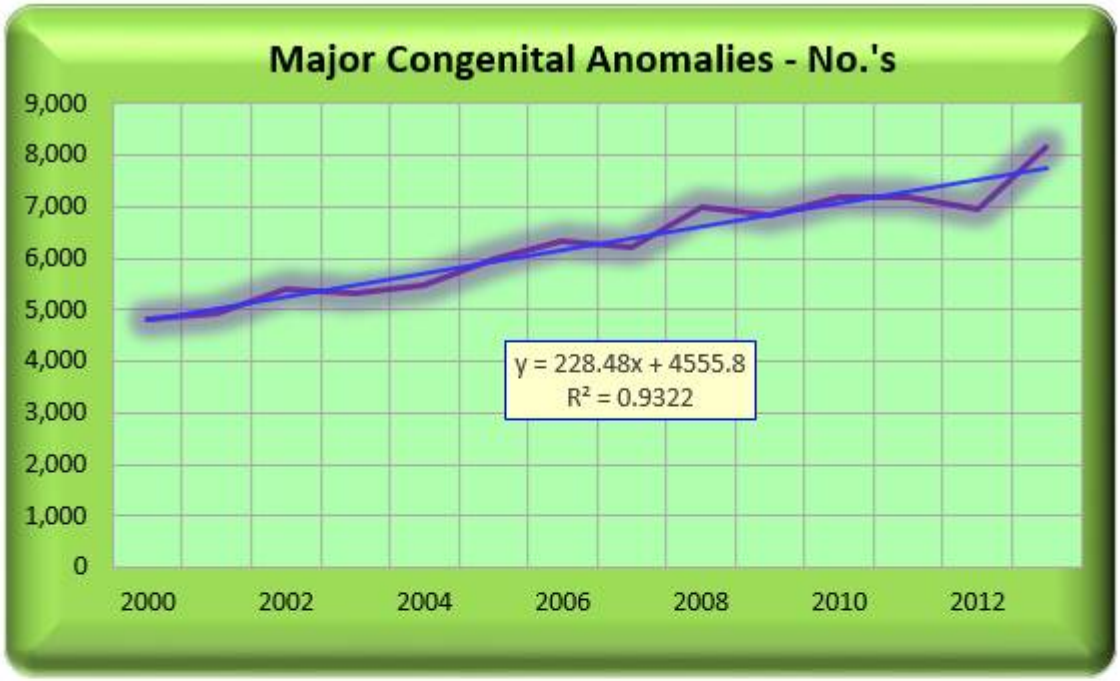
Anomaly	Cumulative Total 2000-2013	Projected Total from Baseline	Excess Above Baseline	% Change 2000-2013	Times (x) Increase Relative to Births
Births	949,317	916,006	33,311	3.6%	1.00
Major Congenital Defects	87,772	67,620	20,152	29.8%	8.20
Major CVS	19,288	14,028	5,260	37.5%	10.31
VSD	4,447	3,794	653	17.2%	4.73
ASD-Secundum	9,833	4,970	4,863	97.8%	26.91
Microcephaly	761	420	341	81.2%	22.33
Chromosomal	3,134	2,450	684	27.9%	7.68

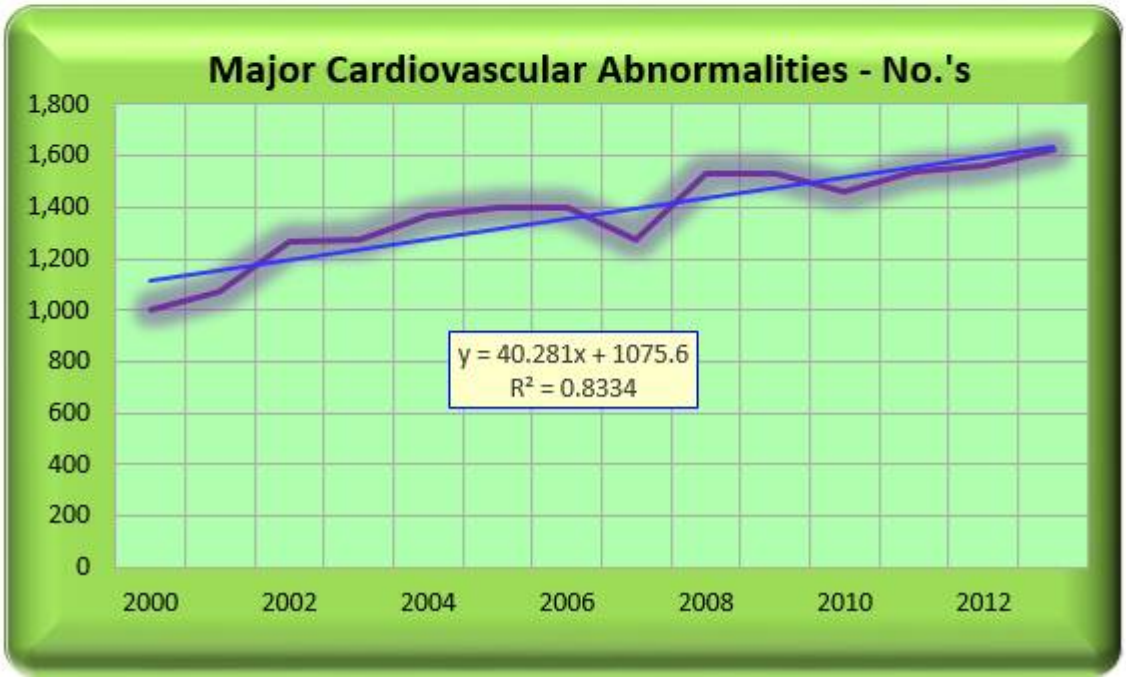
* - From Reference (4)

Figure 1.

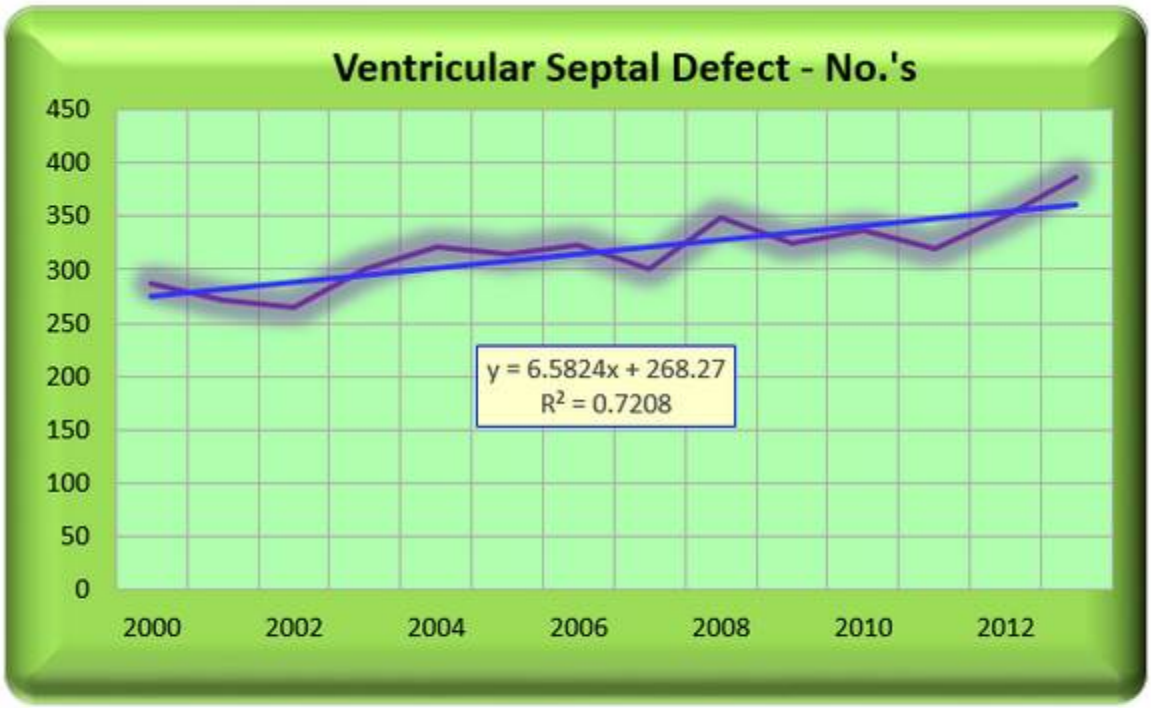

Major Congenital Anomalies - Rates
14%
12%
10%
8%
6%
4%
2%
0%
2000
2002
2004
2006
2008
2010
2012
7.4%
7.9%
7.7%
8.0%
8.7%
8.9%
10.0%
10.0%
10.0%
10.8%
11.0%
10.7%
12.6%

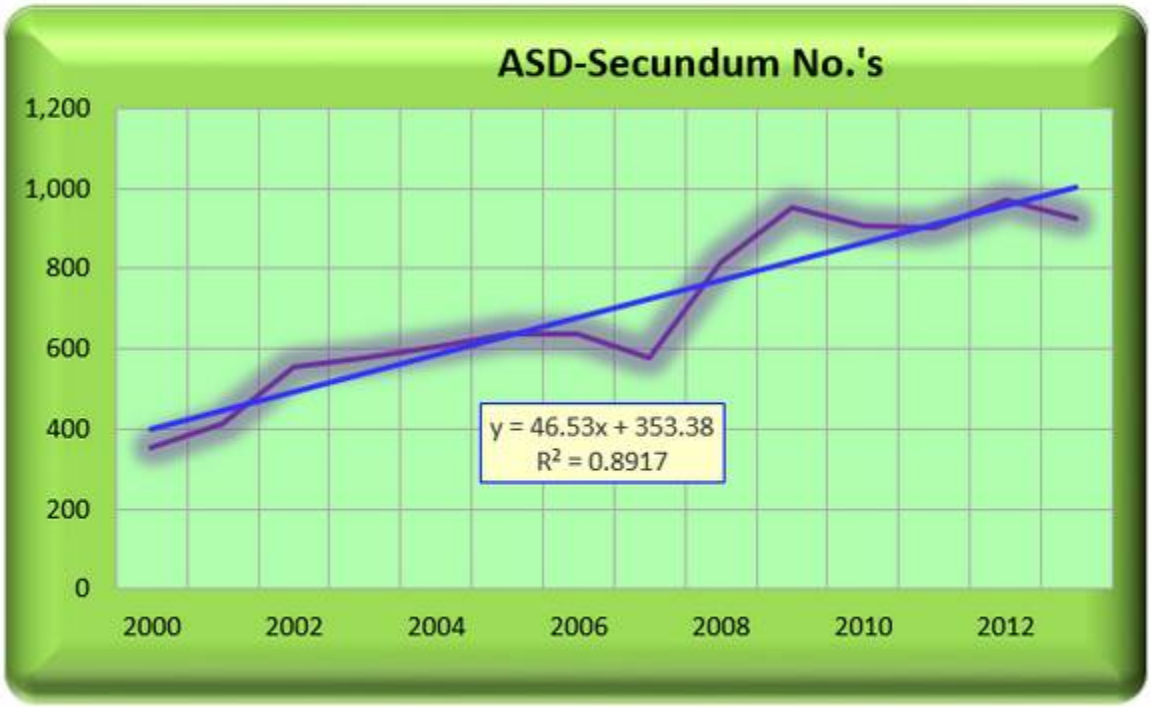
Figures 2, 3.


Major Congenital Anomalies - No.'s
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
0
2000
2002
2004
2006
2008
2010
2012
y = 228.48x + 4555.8
R² = 0.9322

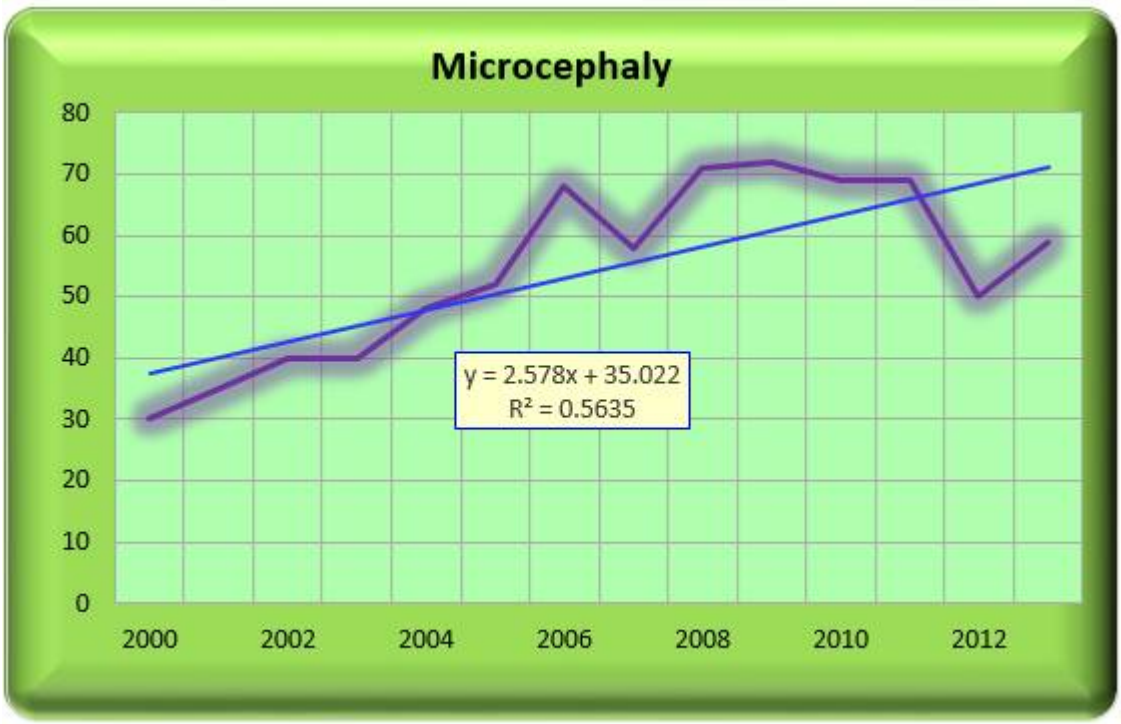

Major Cardiovascular Abnormalities - No.'s
1,800
1,600
1,400
1,200
1,000
800
600
400
200
0
2000
2002
2004
2006
2008
2010
2012
y = 40.281x + 1075.6
R² = 0.8334

Figures 4, 5.


Ventricular Septal Defect - No.'s
450
400
350
300
250
200
150
100
50
0
2000
2002
2004
2006
2008
2010
2012
y = 6.5824x + 268.27
R² = 0.7208


ASD-Secundum No.'s
1,200
1,000
800
600
400
200
0
2000
2002
2004
2006
2008
2010
2012
y = 46.53x + 353.38
R² = 0.8917

Figures 6. 7.


Microcephaly
80
70
60
50
40
30
20
10
0
2000
2002
2004
2006
2008
2010
2012
y = 2.578x + 35.022
R² = 0.5635

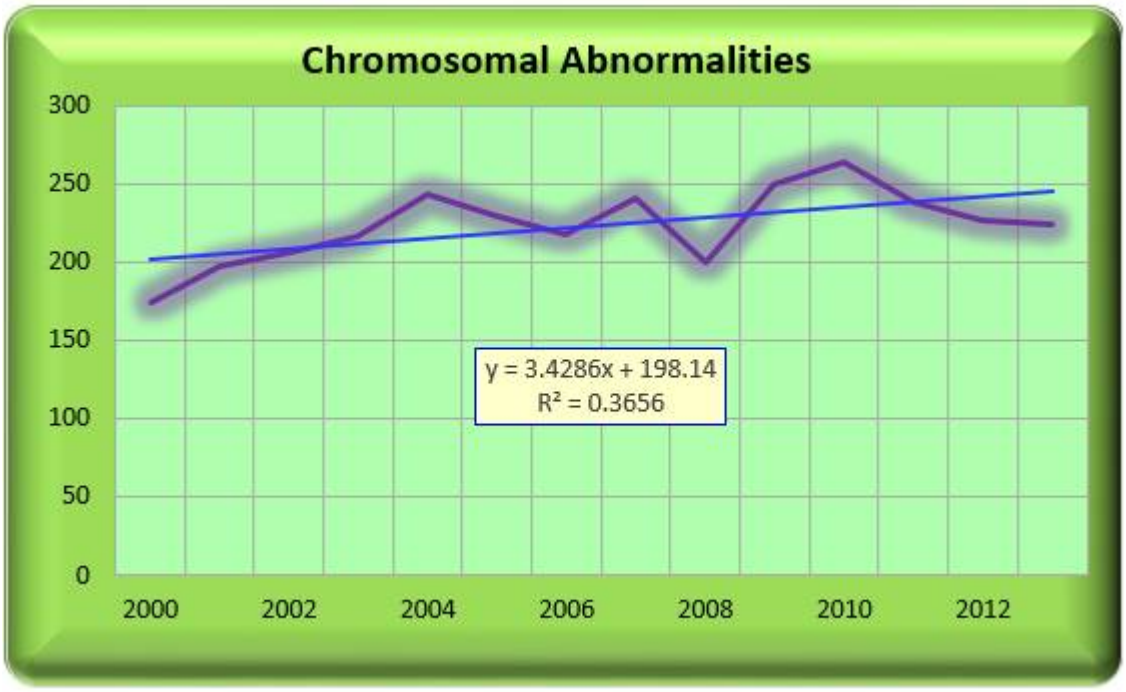
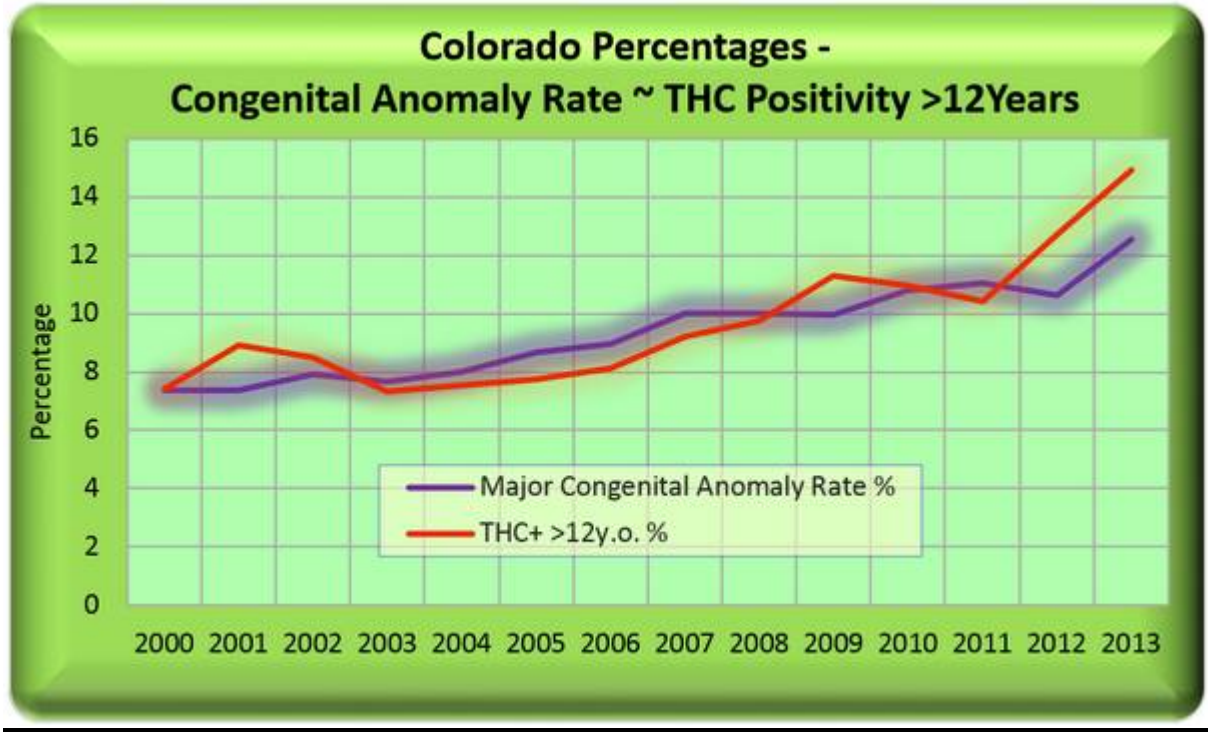

Chromosomal Abnormalities
300
250
200
150
100
50
0
2000
2002
2004
2006
2008
2010
2012
y = 3.4286x + 198.14
R² = 0.3656

Figure 8


Colorado Percentages -
Congenital Anomaly Rate ~ THC Positivity >12Years
Percentage
16
14
12
10
8
6
4
2
0
2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013
Major Congenital Anomaly Rate %
THC+ >12y.o. %

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GENOTOXICITY AND CARCINOGENICITY

Low doses of widely consumed cannabinoids (cannabidiol and cannabidivarin) cause DNA damage and chromosomal aberrations in human-derived cells

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Abstract

Cannabidiol (CBD) and cannabidivarin (CBDV) are natural cannabinoids which are consumed in increasing amounts worldwide in cannabis extracts, as they prevent epilepsy, anxiety, and seizures. It was claimed that they may be useful in cancer therapy and have anti-inflammatory properties. Adverse long-term effects of these drugs (induction of cancer and infertility) which are related to damage of the genetic material have not been investigated. Therefore, we studied their DNA-damaging properties in human-derived cell lines under conditions which reflect the exposure of consumers. Both compounds induced DNA damage in single cell gel electrophoresis (SCGE) experiments in a human liver cell line (HepG2) and in buccal-derived cells (TR146) at low levels (≥0.2 µM). Results of micronucleus (MN) cytome assays showed that the damage leads to formation of MNi which reflect chromosomal aberrations and leads to nuclear buds and bridges which are a consequence of gene amplifications and dicentric chromosomes. Additional experiments indicate that these effects are caused by oxidative base damage and that liver enzymes (S9) increase the genotoxic activity of both compounds. Our findings show that low concentrations of CBD and CBDV cause damage of the genetic material in human-derived cells. Furthermore, earlier studies showed that they cause chromosomal aberrations and MN in bone marrow of mice. Fixation of damage of the DNA in the form of chromosomal damage is generally considered to be essential in the multistep process of malignancy, therefore the currently available data are indicative for potential carcinogenic properties of the cannabinoids.

Keywords CBD · CBDV · Genotoxicity · SCGE assay · MN assay

Abbreviations

BN-MNi	Binucleated cells with micronuclei
CA	Chromosomal aberration
CBD	Cannabidiol
CBDV	Cannabidivarin
CBMN assay	Cytokinesis-block micronucleus assay
CBPI	Cytokinesis-block proliferation index
CT	Cytostasis
CP	Cyclophosphamide
MN	Micronucleus
MNi	Micronuclei
Nbuds	Nuclear buds
NPBs	Nucleoplasmatic bridges
ROS	Reactive oxygen species
SCGE	Single cell gel electrophoresis

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Introduction

Cannabidiol (CBD) and cannabidivarin (CBDV) are naturally occurring cannabinoids which are widely consumed. CBD is structurally related to Δ9-tetrahydrocannabinol (THC) and occurs together with its propyl analogue (CBDV) in *Cannabis sativa* and *C. indica* plants. Both agents cause a variety of pharmacological effects but do not have the

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psychotropic properties which are characteristic for THC. CBD and CBDV are antiepileptic, anticonvulsant, and antipsychotic (Fernández-Ruiz et al. 2013; Hill et al. 2012; Rosenberg et al. 2015; Ujvary and Hanus 2016); furthermore, it was postulated that the former compound prevents inflammation (Borrelli et al. 2009) and may act as an anticarcinogen (Aviello et al. 2012; Massi et al. 2013). Figure 1a–c depict the structure of the compounds.

It was repeatedly stressed that the use of CBD is safe and that it is well-tolerated by humans (Bergamaschi et al. 2011; Iffland and Grotenhermen 2017). At present, a large number of extracts and oils of cannabis plants which contain CBD and CBDV and low levels of THC are marketed in European countries and also in the US, and several clinical trials concerning their health effects are in progress (Fasinu et al. 2016). The preparations are mainly sold via the internet (64%) and in hemp shops (17%), but also in drugstores and pharmacies (Borchardt 2018). The sales of these products are booming at present. According to Forbes Magazine, the market increased by 700% in recent years (http://www.forbes.com) and it is stated in a report of the market intelligence of the Hemp Business Journal that sales will exceed 2.1 Billion USD in 2020 (NSE 2018).

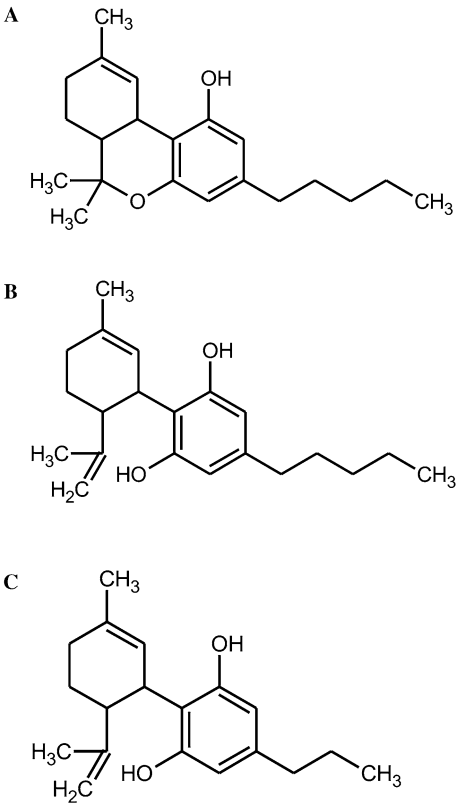

Fig. 1 Chemical structure of the test compounds. **a** Δ9-THC (CAS Nr. 1972-08-3), **b** CBD (CAS Nr. 13956-29-1), **c** CBDV (CAS Nr. 24274-48-4) is a propyl derivative of CBD

Since CBD and CBDV are natural substances, the current legislation does not foresee toxicological testing which is obligatory for pharmaceutical drugs and no potential long-term effects such as induction of cancer, infertility, and malformations in the offspring have been investigated. These latter effects may be due to damage to the genetic material, but only few studies which date back to the 1980s were realized. Zimmerman and Raj (1980) tested CBD in mice and found evidence for induction of micronuclei (MNi) in bone marrow cells of mice, which are formed as a consequence of structural and numerical chromosomal aberrations in bone marrow cells. Furthermore, the same authors reported increased rates of chromosomal aberrations (CA) in the same target tissue by CBD (Zimmerman and Raj 1980).

The aim of the present study was to investigate if CBD and CBDV cause damage to the genetic material in human-derived cells, under conditions which reflect the situation in users. We investigated the effects of these compounds in single cell gel electrophoresis (SCGE) assays which are based on the measurement of DNA migration in an electric field and reflect single and double strand breaks, as well as apurinic sites (Azqueta and Collins 2013). The SCGE technique is among the most widely used methods in genetic toxicology (Neri et al. 2015). The compounds were tested in a human-derived hepatoma cell line (HepG2) which reflects the metabolism of xenobiotics better than other cell lines currently used (Knasmuller et al. 1998). Since CBD and CBDV preparations are mainly consumed orally, additional experiments were conducted with TR146 cells which are derived from the buccal epithelium (Rupniak et al. 1985). To elucidate if (repairable) DNA damage (which is detected in the SCGE experiments) leads to formation of persisting chromosomal mutations, MN cytome experiments were performed, to monitor induction of MNi, which reflect structural and numerical chromosomal aberrations and other nuclear anomalies (Nbuds and NPBs), which are formed as a consequence of gene amplifications and dicentric chromosomes (Fenech 2007).

To characterize the molecular mechanisms, by which the compounds cause genetic instability, additional experiments were performed which enable the assessment of formation of oxidized purines and pyrimidines by use of a modified protocol of the SCGE assay with lesion-specific enzymes according to the protocol of Collins and Dušinská (2002). Finally, a series of experiments with liver homogenate (S9 mix) was conducted to find out if drug-metabolizing enzymes are involved in the activation of the compounds.

Materials and methods

Chemicals

Low melting point agarose (LMPA) and normal melting point agarose (NMPA) were obtained from Gibco (Paisley, UK). Inorganic salts, dimethyl sulfoxide (DMSO), methanol, propidium iodide, hydrogen peroxide, triton X-100, trizma base, bovine serum albumine (BSA), cyclophosphamide, cytochalasin B, Dulbecco's phosphate-buffered saline (DPBS), fetal calf serum (FCS), trypsin–EDTA, Na_2-EDTA, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES), trypan blue and cyclophosphamide (CP) were purchased from Sigma-Aldrich (Steinheim, Germany).

Test compounds

Cannabidiol (CBD, CAS 13956-29-1, purity 99.95%) was obtained from LGC Standards GmbH (Germany) and cannabidivarin (CBDV, CAS 24274-48-4, purity 99.80%) from Sigma-Aldrich (Milan, Italy). Both compounds were dissolved in methanol.

Cultivation of cell lines (HepG2 and TR146)

The human hepatoma cell line (HepG2) was provided by F. Darroudi (Department of Toxicogenetics, Leiden University Medical Centre, the Netherlands). The cells were grown in Eagle's Minimal Essential Medium (EMEM, Sigma-Aldrich, Steinheim, Germany) supplemented with 1.0 mM sodium pyruvate (MNP medium) and 10% FCS. The fifth to eighth passages from stock cultures (in liquid nitrogen) were used for the SCGE and MN experiments.

The human cell line TR146 which is derived from buccal epithelial tissue (Rupniak et al. 1985) was obtained from J. G. Rheinwald (Dermatology Institute of Boston, MA USA). The cells were cultivated in Dulbecco's modified Eagle Medium (DMEM, Sigma-Aldrich, Steinheim, Germany) with 10% FCS. The cells were stored in liquid nitrogen. The fourth to the sixth passage were used for the genotoxicity experiments.

Both cell lines were cultivated under standard conditions (37 °C, humidified atmosphere, 5% CO_2). The media were changed every 2–3 days. When the cultures had reached confluency, the cells were washed with DPBS, detached with trypsin/EDTA, centrifuged and sub-cultured.

Measurements of cytotoxic effects

The viability of the cells was determined with a CASY® cell counter (Schärfe-System GmbH, Reutlingen, Germany). This method is based on the determination of electric potential differences (Lindl et al. 2005). Briefly, cells (2.0×10^5 cells/well) were seeded in 24-wells plates (Becton, Dickinson and Company, NJ, USA) in media which contained different concentrations of CBD (0.22–162 µM) and CBDV (0.66–162 µM) for 3 h or 24 h. In all experiments, solvent controls and positive controls were included. The cells were detached with trypsin–EDTA, centrifuged (200*g*, 5 min, 21 °C) and suspended in 1.0 mL medium. 50.0 µL of these suspensions were transferred to CASY-cups (OLS OMNI Life Science GmbH & Co. KG, Bremen, Germany). For each experimental point, two independent experiments were performed and means ± standard deviations were calculated. Additionally, we tested the viability of the cells after exposure to the test compounds with the trypan blue exclusion technique (Lindl and Bauer 1994).

Single cell gel electrophoresis (SCGE) assays (standard conditions)

The experiments were conducted according to the protocol of Tice et al. (2000) under alkaline conditions. Only cultures with a viability ≥ 80% were evaluated in SCGE assays.

The indicator cells (2.0×10^5 cells/well) were transferred into 24-well plates which contained 1.0 mL medium with different concentrations of CBD and CBDV. The cells (HepG2) were exposed to the test compounds for 3 h and 24 h (3 h: dose range 0.66–54, 24 h: dose range 0.22–18 µM). TR146 cells were treated with the cannabinoids for 3 h (dose range 2.00–54 µM). In all experiments, solvent controls (methanol) and positive controls (H_2O_2, 50 µM) were included. The pellets were resuspended in low melting point agarose (0.5% LMPA). Subsequently, the cells were spread on pre-coated agarose slides (1.5% NMPA) and lysed in the dark at 4 °C for at least 60 min. After 30 min of unwinding under alkaline conditions (pH > 13), electrophoresis was carried out for 30 min (300 mA, 1.0 V/cm, at 4 °C); neutralization was performed twice for 8 min. Air-dried slides were stained with propidium iodide (10 µg/mL). Subsequently, the percentage of DNA in the tails was measured by use of an image analysis system (Comet IV, Perceptive Instruments Ltd., Burry St. Edmunds', UK). For each experimental point, two slides were prepared and 50 nuclei were evaluated randomly on each slide. Two independent experiments were performed.

In experiments with rat liver homogenate (S9), 10 µL S9 mix was added to the inoculation mix (final protein concentration 30 mg/mL). MUTAZYME™ rat S9 mix (10%) was purchased from TrinovaBiochem GmbH (Giessen, Germany). MUTAZYME™ consists of Aroclor 1254-induced male Sprague Dawley rat liver S9 which was lyophilized with NADP, D-glucose-6-phosphate, $MgCl_2$/KCl in pH 7.4 sodium phosphate buffer. The mixtures were incubated for 3 h (37 °C; shaking 250 rpm). Subsequently, the cells were

washed and processed as described above. Two independent experiments were performed. For each experimental point, two slides were prepared and 50 nuclei were evaluated randomly from each slide.

Single cell gel electrophoresis (SCGE) assays with lesion-specific enzymes

The impact of the drugs on the formation of oxidized DNA bases was monitored in additional experiments with lesion-specific enzymes. Formamidopyrimidine DNA glycosylase (FPG) and endonuclease III (ENDO III) were purchased from Sigma-Aldrich (Steinheim, Germany). To define the optimal concentrations of the enzymes, calibration experiments were carried out before the main experiments [for details see Collins et al. (1997), data not shown].

The cells (HepG2) were exposed to the test compounds as described above. The experiments with lesion-specific enzymes were conducted according to the protocol of Collins and Dusinska (2002).

After lysis, the slides were washed for 8 min twice with enzyme reaction buffer (40 mM HEPES, 0.1 M KCl, 0.5 mM Na_2EDTA, 0.2 mg/mL BSA, pH 8.0). Subsequently, the nuclei were treated either with 50 µL of the enzyme solutions or with the enzyme buffers. The incubation time for experiments with FPG was 30 min and for Endo III 45 min at 37 °C, respectively. After the treatment, electrophoresis was carried out under standard conditions (30 min, 300 mA, 1.0 V/cm, at 4 °C, pH > 13). After electrophoresis, the slides were processed and evaluated as described above. Two independent experiments were performed. For each experimental point, two cultures were set up. From each culture, two slides were prepared and 50 cells were evaluated from each slide.

Cytokinesis-block micronucleus (CBMN) assays with HepG2

The experiments were conducted as described by Koller et al. (2014). Briefly, 5.0×10^5 cells/well were seeded in 6-well plates with 3.0 mL medium and allowed to attach overnight. Subsequently, the medium was removed after washing with DPBS. The cells were treated with different concentrations (0.07–2 µM) of the test compounds in serum-free medium for 3 h. Cyclophosphamide (final concentration 500 µg/mL) was used as a positive control. After treatment of the cells with the drugs for 3 h, they were washed with PBS. Subsequently, they were incubated with cytochalasin B (3.0 µg/mL) to block cytokinesis and DMEM (with 10% FCS) for 27–28 h. Then, the cells were washed, trypsinized and harvested. Slides were prepared with the cyto-centrifugation method (Fenech 2007). After drying, they were stained with Diff Quick (Dade Behring, Deerfield, IL, USA) and fixed with Entellan (Sigma-Aldrich, Steinheim, Germany).

Per experimental point, two cultures were made. Four slides were prepared and 2000 cells were evaluated. Different endpoints were scored namely, mono-nucleated, binucleated (BN) and multi-nucleated cells as well as the rates of binucleated cells with MN (BN–MN), the total number of MN in binucleated cells (MNi), nuclear buds (Nbuds), and nucleoplasmatic bridges (NPBs). The cytokinesis-block proliferation indices (CBPI) were calculated with 500 cells according to the formula $CBPI = [M1 + 2M2 + 3(M3 + M4)]/N$ (N is the total number of scored cells), $M1$–$M4$ refers to the number of cells with one to four nuclei (OECD 2016). The toxicity of the compounds was indirectly assessed by the assumption that a CBPI of 1.0 corresponds to 100% cytotoxicity (OECD 2016). Five concentrations of each drug were used to determine the CBPI values. Two independent experiments were performed; per experimental point, four slides were prepared and 2000 cells were evaluated. In agreement with OECD guideline #487 (OECD 2016), only doses causing less than 60% cytotoxicity were analyzed with regard to formation of nuclear anomalies. Early necrotic cells, characterized by pale cytoplasm and presence of many vacuoles, and late necrotic cells, identified by loss of cytoplasm and damaged nuclear membranes, were scored according to the protocol of Fenech (2007). Apoptotic cells were identified morphologically by changes in the chromatin structure and by nuclear fragmentation (Fenech 2007).

Statistical analyses

All results were analyzed with the GraphPad Prism 5 software system (LaJolla, CA, USA). The data from the SCGE experiments and from the MN assays are presented as means ± SD. The results of CBMN and SCGE assays (under standard conditions and after treatment with lesion-specific enzymes) were analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test. The t test was used for experiments with/without S9 in TR146 cells to calculate the statistical differences between the groups after the treatment of the cells with both compounds. Differences were considered as significant when the p values were ≤ 0.05.

All statistical calculations are based on comparisons between results which were obtained with cells which had been treated with the test compounds and results which were obtained with corresponding solvent controls.

Results

Cytotoxic effects of test compounds

Since cytotoxic effects may lead to false positive results in SCGE assays (Henderson et al. 1998), several experimental series were conducted with HepG2 and TR146 cells, in which the indicator cells were exposed to different

concentrations of CBD and CBDV. The results of these experiments are summarized in Figures S1 and S2 (supplementary information). It can be seen that the viability of the HepG2 was not affected when the cells were exposed to concentrations ≤ 54 µM for 3 h; the highest dose (162 µM) caused a clear effect, and the viability of the cells decreased by approximately 50%. When the treatment time was extended to 24 h, a decline of viable cells was also seen with 54 µM (Fig. S1A–D). The impact of the compounds on the viability of TR146 cells is shown in Figures S2A-B.

The vitality of the HepG2 cells in SCGE experiments was also determined with the trypan blue exclusion technique after treatment with 54 µM of CBD and CBDV (the highest dose tested in SCGE experiments) and was 90% ± 8 and 95% ± 4, respectively. The corresponding values for TR-146 cells are 91% ± 5 and 87% ± 4 (numbers indicate values obtained with three cultures ± standard deviations). Since misleading/false positives may occur in SCGE experiments only when the viability of the cells declines below 80% (Henderson et al. 1998), it can be excluded that the results which we obtained in the SCGE tests are due to acute toxic effects.

SCGE assays with HepG2 and TR146 (standard conditions)

The results of SCGE experiments with the cannabinoids are summarized in Figs. 2, 3 and 4. Results of individual experiments can be found in supplementary tables SI 1A-B. Since it is known that the genotoxic response of promutagens in HepG2 may increase after extended treatment (Natarajan and Darroudi 1991), two exposure periods (3 h and 24 h) were tested. Both drugs caused DNA damage in both cell types (HepG2 and TR146). In the liver-derived cells, significant induction of damage was seen with both compounds at concentrations ≥ 6.0 µM after 3 h (Fig. 2a, b). When the cells were treated for 24 h, clear damage was observed with the lower concentrations (≥ 2.0 µM) (Fig. 2c, d).

Also with TR146 cells, which are derived from the buccal cavity, positive findings were obtained under identical conditions, i.e., induction of comets was detected with both drugs at concentrations ≥ 6.0 µM after 3 h (Fig. 3a, b).

It is notable that CBD was more active than its propyl analogue (CBDV) in both cell lines, when the cells were exposed for 3 h, i.e., the extent of DNA damage which was seen with the former compound under identical conditions was approximately threefold higher.

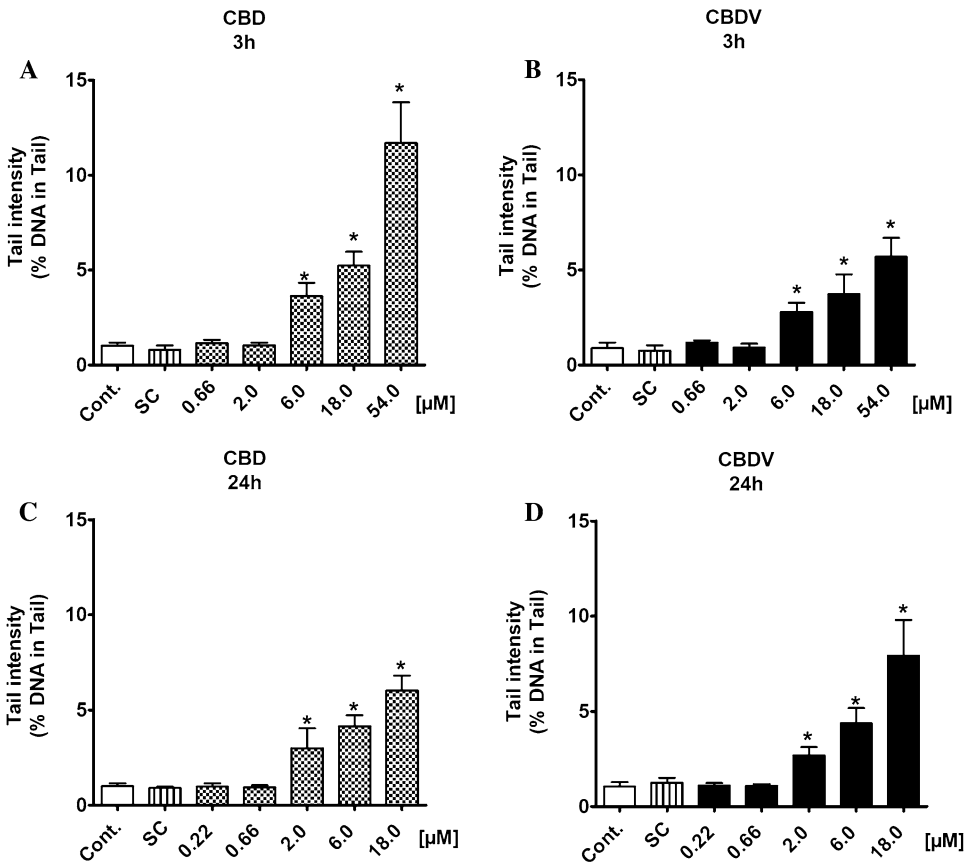

Fig. 2 **a, b** Induction of DNA damage by CBD and CBDV in a human-derived liver cell line (HepG2). The cells were treated with different concentrations of the test compounds for 3 and 24 h. Methanol was used as a solvent control [for 3 h CBD: 1.70% (v/v) and CBDV: 1.55% (v/v); for 24 h CBD: 0.56% (v/v) for CBDV: 0.52% (v/v)]. Hydrogen peroxide (50 µM) was used as a positive control (the cells were treated for 5 min on ice) and induced clear positive effects (26.57 ± 3.64% DNA in tail). Bars indicate means ± SD of results obtained with two parallel cultures per experiment (from each culture two slides were made and 50 cells were evaluated per slide). Stars indicate statistical significance ($p \leq 0.05$, ANOVA). All statistical calculations are based on comparisons between results which were obtained with cells which had been treated with the test compounds and results which were obtained with corresponding solvent controls

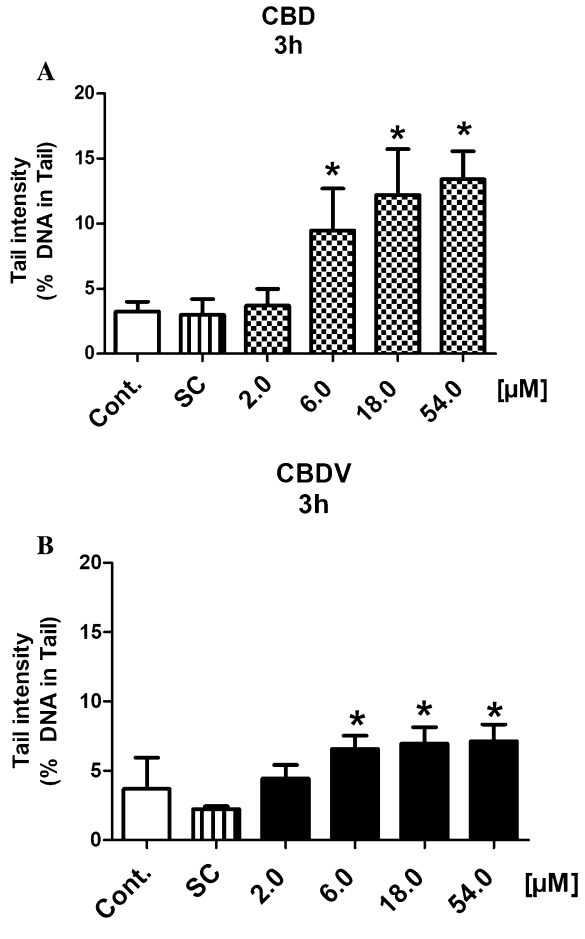

Fig. 3 **a, b** Induction of DNA damage by CBD and CBDV in a human-derived buccal cell line (TR146). The cells were treated with different concentrations of the test compounds for 3 h. Methanol was used as solvent control [CBD: 1.70% (v/v) and CBDV: 1.55% (v/v)]. Hydrogen peroxide (50 µM) was used as a positive control (the cells were treated for 5 min on ice). The peroxide induced clear positive effects (20.12 ± 1.84% DNA in tail). Bars indicate means ± SD of results obtained with two parallel cultures per experiment (from each culture two slides were made and 50 cells were evaluated per slide). Stars indicate statistical significance ($p \leq 0.05$, ANOVA). All statistical calculations are based on comparisons between results which were obtained with cells which had been treated with the test compounds and results which were obtained with corresponding solvent controls

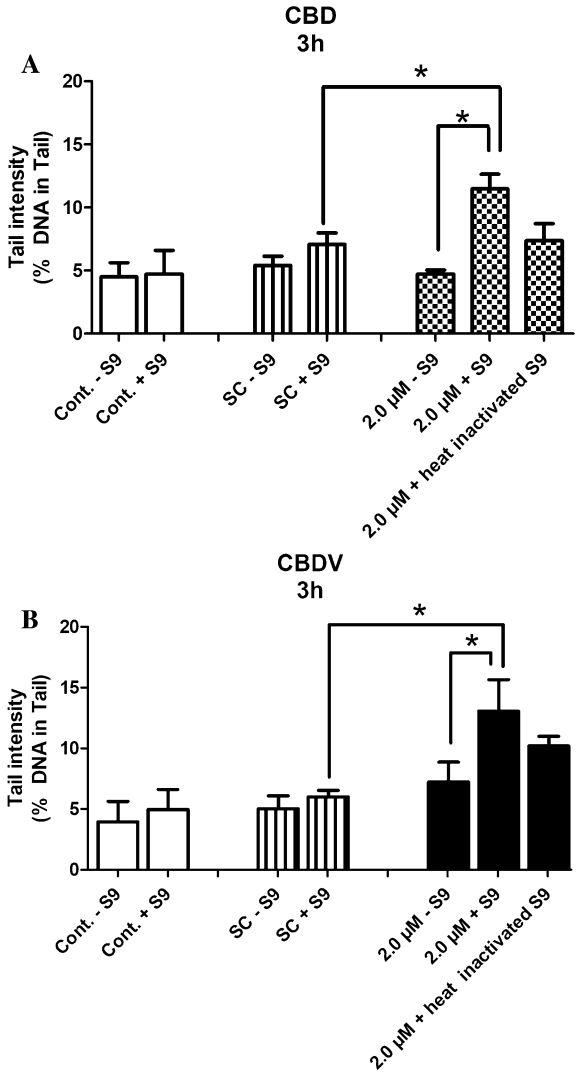

Fig. 4 **a, b** Impact of liver enzyme homogenate on the DNA-damaging activity of CBD and CBDV in TR146 cells. The cells were treated with 2.0 µM of the cannabinoids and in parallel with liver enzyme homogenate (for details see "Materials and methods"). Bars indicate means ± SD of results obtained with two parallel cultures per experiment (from each culture two slides were made and 50 cells were evaluated per slide). Stars indicate statistical significance ($p \leq 0.05$, Two-tailed paired *t* test). All statistical calculations are based on comparisons between results which were obtained with cells which had been treated with the test compounds and results which were obtained with corresponding solvent controls

To find out if the compounds are converted to mutagenic metabolites by liver enzymes, an additional experimental series was realized, in which S9 mix (which contains active phase I enzymes) was added to the incubation during the treatment of TR146 cells with the cannabinoids. The results are shown in Fig. 4a, b. Addition of the enzyme homogenate caused induction of DNA damage in TR146 cells, but no such effect was seen when the liver enzymes were inactivated by heating.

SCGE assays with lesion-specific enzymes with HepG2

To elucidate if the drugs cause oxidative damage of DNA bases, experiments were conducted with lesion-specific enzymes (FPG and ENDO III). The results are summarized in Figs. 5a, b and 6a, b.

It is evident that CBD and CBDV cause oxidation of purines and pyrimidines. Even with the lowest levels

(0.66 µM), significant induction of comet formation was observed.

Cytokinesis-block micronucleus (CBMN) assays with HepG2

To find out if treatment of human liver-derived cells leads to formation of MNi, which reflect structural and numerical chromosomal aberrations, cytome MN experiments were conducted with HepG2 cells. The results are summarized in Table 1. Data from individual experiments can be found in supplementary tables SI 2A-B.

Both compounds caused induction of MNi at low concentrations (≥0.22 µM). Additionally, a significant increase of other nuclear anomalies (Nbuds and NPBs), as well as induction of cell death (necrosis and apoptosis) was observed after treatment with both drugs.

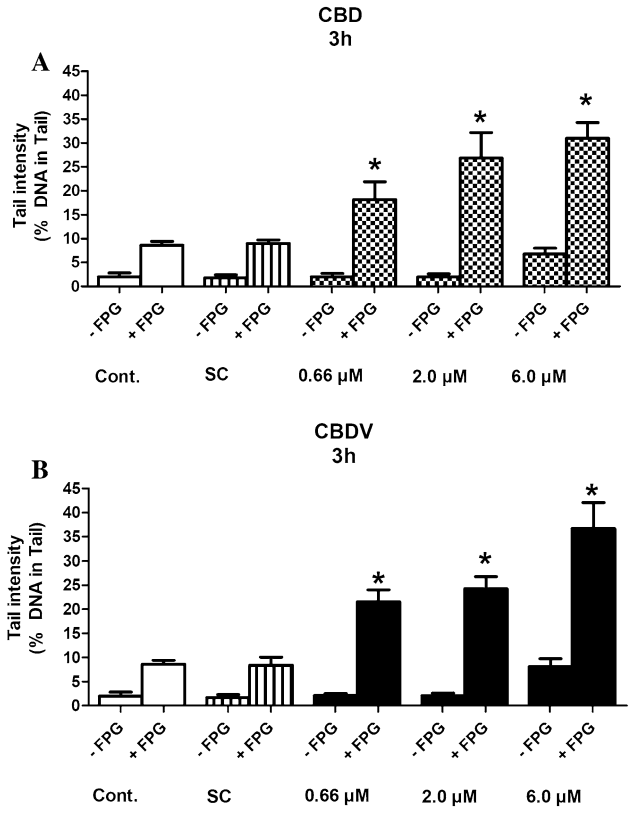

Fig. 5 **a, b** Formation of oxidized purines in HepG2 cells by CBD and CBDV. The cells were exposed to the test compounds for 3 h. Subsequently, the nuclei were isolated after lysis and treated with FPG or with the corresponding buffers before electrophoresis for 30 min. Bars indicate means ± SD of results obtained with two cultures per experimental point. From each culture, two slides were made and 50 cells were evaluated per slide. Stars indicate statistical significance ($p \leq 0.05$, ANOVA). All statistical calculations are based on comparisons between results which were obtained with cells which had been treated with the test compounds and results which were obtained with corresponding solvent controls

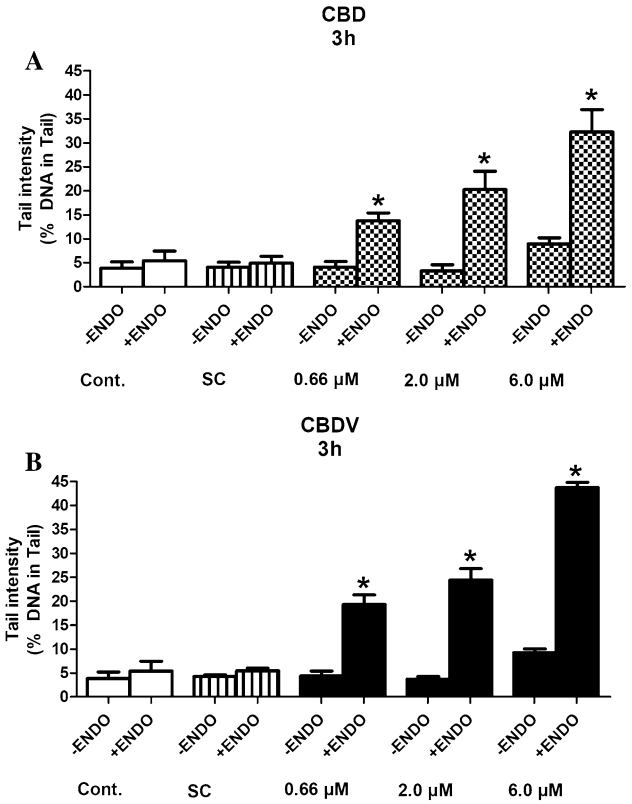

Fig. 6 **a, b** Formation of oxidized pyrimidines in HepG2 cells by CBD and CBDV. The cells were exposed to the test compounds for 3 h. Subsequently, the nuclei were isolated after lysis and treated with ENDO III or with the corresponding buffers before electrophoresis for 45 min. Bars indicate means ± SD of results obtained with two cultures per experimental point. From each culture, two slides were made and 50 cells were evaluated per slide. Stars indicate statistical significance ($p \leq 0.05$, ANOVA). All statistical calculations are based on comparisons between results which were obtained with cells which had been treated with the test compounds and results which were obtained with corresponding solvent controls

Discussion

The results of the present study show that CBD and CBDV cause formation of comets (which reflect single and double strand breaks and apurinic sites), oxidation of DNA bases and induction of MN (which are formed as a consequence of structural and numerical chromosomal aberrations).

The effects were seen at concentrations which are in the range of the levels also found in the blood of users. The highest concentrations of CBD detected after smoking were between 0.25 and 2.18 µM in plasma (Haney et al. 2016; Ohlsson et al. 1986). Cells in the oral cavity of users who consume oils, sprays or smoke dried plant material may be exposed to much higher doses, but no experimental data are currently available according to our knowledge. For CBDV, exposure data from humans are missing. As shown in Table 1, we found significant induction of MN

Table 1 Impact of the two cannabinoids on MN formation and on the rates of various nuclear aberrations in HepG2 cells

Compounds	Concentrations (µM)	CPBI Mean ± SD	CT %	BN-MN[a] Mean (‰) ± SD	MNi[b] Mean (‰) ± SD	Nbuds Mean (‰) ± SD	NPBs Mean (‰) ± SD	Necrosis Mean (‰) ± SD	Apoptosis Mean (‰) ± SD
Neg. Ctrl	0	2.04 ± 0.03	–	5.25 ± 0.35	5.75 ± 0.35	4.75 ± 0.35	3.50 ± 0.71	6.25 ± 0.35	3.00 ± 0.71
CBD	0.07	2.00 ± 0.08	3.92	6.50 ± 1.41	6.50 ± 1.41	16.00 ± 2.12*	5.25 ± 0.35	16.25 ± 1.77*	13.50 ± 0.71*
	0.22	1.93 ± 0.04	10.60	21.00 ± 1.41*	31.00 ± 2.12*	25.50 ± 2.83*	8.50 ± 1.41*	21.00 ± 0.70*	25.25 ± 3.18*
	0.66	1.83 ± 0.04	20.22	31.25 ± 2.47*	46.25 ± 3.89*	37.25 ± 1.06*	10.00 ± 1.41*	30.75 ± 1.77*	29.00 ± 1.41*
	2.00	1.72 ± 0.01	30.76	39.25 ± 3.89*	53.25 ± 2.47*	43.00 ± 2.83*	14.00 ± 0.71*	33.50 ± 2.12*	37.25 ± 1.77*
SC[c]		1.80 ± 0.00	23.05	5.00 ± 1.41	6.25 ± 0.35	5.50 ± 1.41	3.25 ± 1.06	6.75 ± 1.06	3.00 ± 0.71
CBDV	0.07	1.95 ± 0.05	9.17	6.00 ± 0.71	6.00 ± 0.71	15.25 ± 1.77*	6.00 ± 2.12	15.25 ± 2.47*	13.75 ± 1.77*
	0.22	1.93 ± 0.04	10.60	26.00 ± 2.83*	29.75 ± 1.77*	36.25 ± 3.18*	10.00 ± 0.71*	18.50 ± 1.41*	21.75 ± 1.06*
	0.66	1.79 ± 0.01	24.03	32.00 ± 0.71*	45.50 ± 1.41*	40.00 ± 2.12*	13.25 ± 1.77*	24.5 ± 1.41*	28.75 ± 3.89*
	2.00	1.77 ± 0.03	25.97	41.25 ± 2.47*	51.25 ± 3.89*	45.75 ± 2.47*	16.00 ± 2.12*	34.75 ± 2.47*	30.00 ± 2.83*
SC[c]		1.81 ± 0.02	22.54	5.00 ± 00	5.75 ± 0.35	5.00 ± 0.71	3.25 ± 0.35	6.25 ± 1.06	3.00 ± 0.71
Pos. Ctrl	500 µg/mL	1.80 ± 0.01	23.54	42.25 ± 5.30*	56.75 ± 1.06*	35.50 ± 1.41*	11.75 ± 1.06*	16.25 ± 1.77*	9.25 ± 3.18

CBPI cytokinesis-block proliferation indices, *CT* cytostasis (%), HepG2 cells were treated with different concentrations of the test compounds for 3 h. Numbers represent results (means ± SD) obtained in two independent experiments, and in each experiment, two cultures were made per experimental point. Four slides were prepared and 2000 cells were evaluated. All statistical calculations are based on comparisons between results which were obtained with cells which had been treated with the test compounds and results which were obtained with corresponding solvent controls.

BN–MNi binucleated cells with micronuclei, *MNi* micronuclei, *Nbuds* nuclear buds, *NPBs* nucleoplasmatic bridges, *Neg. Ctrl* cells cultivated in medium, *SC* solvent control, *Pos. Ctrl* cyclophosphamide (500 µg/ml)

*Significant differences from solvent control values (Dunnett test, $p \leq 0.05$)

[a]Number of binucleated cells with MN

[b]Total number of MN from binucleated cells

[c]Methanol was used as solvent control [0.06% (v/v) in experiments with CBD and 0.05% (v/v) in experiments with CBDV]

with both compounds after treatment of the cells with concentrations ≥ 0.22 µM in the present study. Furthermore, increased rates of NBuds and NPBs, which are formed as a consequence of gene amplification and dicentric chromosomes (Fenech 2007), were also detected under identical conditions.

As described in the introduction, results of older studies are available (when no CBD-containing preparations were sold on the market). They show that CBD causes induction of MN and CA in bone marrow of mice (Zimmerman and Raj 1980), while no positive results were obtained in unscheduled DNA synthesis (UDS) experiments with fibroblasts in vitro (Zimmerman et al. 1978). MN induction was found in three independent experimental series after i.p. administration of CBD; the test was in partial agreement with the U.S. EPA guidelines (Mavournin et al. 1990; OECD 2016), i.e., several doses were tested, five animals were used per group, a sufficient number of cells was evaluated and positive/negative controls were included. However, the impact of the drug on erythropoiesis, which may lead to false results and OECD #474 (Tweats et al. 2007) was not taken into account. The evidence for induction of MN is supported by results of chromosomal analyses of metaphase cells from the bone marrow which showed that i.p. administration of 10 mg/kg caused a sevenfold increase over the background (Zimmerman and Raj 1980).

The only SCGE result with CBD was published by Aviello et al. (2012) who conducted a single dose experiment with colon-derived (CaCo2) cells. The authors found no induction of DNA damage when the cells were treated with 10 µM CBD for 24 h. We did not find any results of mutagenicity studies with CBDV in the literature, while several investigations were conducted with THC which is structurally related to both compounds (Fig. 1). Consistently negative results were obtained in microbial experiments and in in vitro studies with mammalian cells and human leukocytes (Neu et al. 1970; Stenchever and Allen 1972; Stoeckel et al. 1975; Zimmerman et al. 1978), while studies done with laboratory rodents yielded controversial findings (Stoeckel et al. 1975; Van Went 1978). In a human study, clear induction of chromosomal aberrations was found in lymphocytes of individuals who consumed the alkaloid orally (Nichols et al. 1974).

The results of experiments with lesion-specific enzymes (Figs. 5a, b, 6a, b) show that both compounds cause oxidative damage of purines and pyrimidines. In this context, it is notable that pro- as well as antioxidant effects of CBD have been described. For example, the neuroprotective

effects of CBD towards alcohol-induced toxicity were attributed to its antioxidant properties (Hamelink et al. 2005). Protective effects seen in LPS-stimulated macrophages were explained by inhibition of formation of pro-inflammatory cytokines, which cause formation of free oxygen radicals (Rajan et al. 2016). A molecular explanation for the antioxidant properties of CBD can be found in a publication of Borges et al. (2013). On the other hand, it was shown that CBD induces oxidative stress via activation of caspase-8 leading to apoptosis (Wu et al. 2008). Furthermore, induction of cyclooxygenase 2 (COX-2) was found in Zucker diabetic fatty rats, which leads to formation of pro-inflammatory prostaglandins and reactive oxygen species (ROS) (Wheal et al. 2014).

Our results with liver enzyme homogenate (Fig. 4) suggest that drug-metabolizing enzymes (in particular CYPs which are contained in the enzyme mix) increase the genotoxic properties of CBD and CBDV. It is well-documented, that different CYPs (in particular CYP1A1, 1A2 and 3A4) catalyze the formation of hydroxyl derivatives of CBD (Ujvary and Hanus 2016), but the mutagenic properties of these metabolites have not been investigated so far.

The most relevant result of the present investigation is the detection of MN induction by CBD and CBDV at low, physiologically relevant concentrations. MNi are formed as a consequence of chromosomal damage and it is well-documented, that increased rates in lymphocytes of humans are indicative for cancer risks (Bonassi et al. 2007). The results of the present experiments and also the findings of Zimmerman and Raj (1980), who found induction of MN and CA in vivo in bone marrow of mice, indicate that CBD is a potent mutagen. The International Committee on Harmonized Guidance on Genotoxicity Testing of Pharmaceuticals states in a position paper very clearly that "unequivocally genotoxic compounds in the absence of other data are presumed to be trans-species carcinogens, implying a hazard in humans. Such compounds need to be subjected to long-term carcinogenicity studies" (Muller et al. 1999). Furthermore, it should be also explored if sperm abnormalities, which may be also caused by genomic instability and were induced by CBD in mice (Zimmerman and Zimmerman 1990), are due to DNA damage and may lead to infertility of users. As mentioned above, no data from long-term carcinogenicity experiments with rodents are available at present. It is notable in this context that it was found that the sensitivity of a combination of positive MN assays with rodents and in vitro SCGE assays for the detection of group 1 carcinogens (IARC) was found to be 95.6% (Bhagat 2018). In regard to the MN data obtained in bone marrow cells, it will be relevant to investigate if the drugs induce alterations of the erythropoetic system (see above) and also if inhalative and oral exposure cause adverse effects. Additional experiments to elucidate the molecular mechanisms by which the cannabinoids cause damage of the genetic material would also contribute to a better understanding of their possible health risks in humans.

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Compliance with ethical standards

Conflict of interest The authors state that they have no conflict of interest.

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Risk of Selected Birth Defects with Prenatal Illicit Drug Use, Hawaii, 1986–2002

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The literature on the association between prenatal illicit drug use and birth defects is inconsistent. The objective of this study was to determine the risk of a variety of birth defects with prenatal illicit drug use. Data were derived from an active, population-based adverse pregnancy outcome registry. Cases were all infants and fetuses with any of 54 selected birth defects delivered during 1986–2002. The prenatal methamphetamine, cocaine, or marijuana use rates were calculated for each birth defect and compared to the prenatal use rates among all deliveries. Among all deliveries, the prenatal use rate was 0.52% for methamphetamine, 0.18% for cocaine, and 0.26% for marijuana. Methamphetamine rates were significantly higher than expected for 14 (26%) of the birth defects. Cocaine rates were significantly higher than expected for 13 (24%) of the birth defects. Marijuana rates were significantly higher than expected for 21 (39%) of the birth defects. Increased risk for the three drugs occurred predominantly among birth defects associated with the central nervous system, cardiovascular system, oral clefts, and limbs. There was also increased risk of marijuana use among a variety of birth defects associated with the gastrointestinal system. Prenatal uses of methamphetamine, cocaine, and marijuana are all associated with increased risk of a variety of birth defects. The affected birth defects are primarily associated with particular organ systems.

It is estimated that hundreds of thousands of women use illicit drugs during pregnancy each year in the United States (Hutchins, 1997). Studies have varied widely in the reported prevalence of illicit drug use during pregnancy due to differences in population size, population studied, and study design (Derauf et al., 2003; Norton-Hawk, 1997). Prenatal illicit drug use has been associated with preterm delivery; decreased birth weight, length, and head circumference; and adverse neurobehavioral characteristics shortly after birth, such as withdrawal symptoms (e.g., irritability, tremors, and feeding problems) (Behnke et al., 2001; Cosden et al., 1997; Holzman & Paneth, 1994; Ostrea et al., 1992; Chouteau et al., 1988; Little et al., 1988).

Studies that examine the impact of illicit drug use during pregnancy are often subject to certain limitations (Cosden et al., 1997; Hutchins, 1997; Norton-Hawk, 1997). Individuals who use one illicit drug frequently use other illicit drugs. Thus it is difficult to elicit whether the observed effects are due to a specific drug. Similarly, woman who use illicit drugs during pregnancy may also have other adverse health behaviors or inadequate prenatal care that could account for the observed outcomes.

Another difficulty is the identification of the illicit drug exposure. The two main methods for identification of illicit drug use are through self-report or through toxicology tests, neither of which is ideal. Individuals might be reluctant to report illicit drug use because of the negative moral connotations associated with the practice as well as potential legal ramifications. For the same reasons, individuals may be reluctant to undergo toxicology tests. Furthermore, toxicology tests only provide information on recent illicit drug use. Since both methods of identifying illicit drug exposure have limitations and one may not be superior to the other, it was suggested that both be used together in order to obtain a more accurate estimate of illicit drug use (Christmas et al., 1992).

A number of studies investigated whether prenatal illicit drug use causes birth defects. Various studies reported that maternal cocaine use increased risk of microcephaly, cardiac defects, situs inversus, ventricular septal defect, atrial septal defect, endocardial cushion defect, genitourinary defects, and gastroschisis (Abe et al., 2003; Ferencz et al., 1997a, 1997b, 1997c; Battin et al., 1995; Torfs et al., 1994; Lipshultz et al., 1991; Martin & Edmonds, 1991). Prenatal

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marijuana use was associated with ventricular septal defect, Ebstein anomaly, gastroschisis, and limb–body wall complex (Wlliams et al., 2004; Luehr et al., 2002; Ferencz et al., 1997e; Correa-Villasenor et al., 1994; Torfs et al., 1994). Maternal methamphetamine or amphetamine use has been reported to increase risk of cardiac defects, musculoskeletal defects, and gastroschisis (McElhatton et al., 2000; Torfs et al., 1994). However, other research observed no association between birth defects and maternal use of illicit drugs in general (Frey & Hauser, 2003; Hussain et al., 2002; Croen et al., 2000; Penman et al., 1998; Li et al., 1995), cocaine (Kuehl & Loffredo, 2002; Beaty et al., 2001; Behnke et al., 2001; Gardner et al., 1998; Ferencz et al., 1997d; Hume et al., 1997; Shaw et al., 1996; Martin & Khoury, 1992; Martin et al., 1992; Adams et al., 1989), marijuana (Steinberger et al., 2002; Beaty et al., 2001; Ferencz et al., 1997d; Shaw et al., 1996; Adams et al., 1989), or methamphetamine or amphetamine (Shaw et al., 1996; Little et al., 1988).

Much of the published research on prenatal illicit drug use and birth defects were case reports, involved a small number of cases, were not population-based, or focused on only one or a few particular birth defects. The intent of the current investigation was to evaluate the relationship between use of methamphetamine, cocaine, and marijuana during pregnancy and a variety of birth defects using population-based data from over 300,000 live births.

METHODS

This retrospective study used data from the Hawaii Birth Defects Program (HBDP), a statewide, population-based registry for adverse pregnancy outcomes (National Birth Defects Prevention Network, 2004). The HBDP includes all infants and fetuses of any pregnancy outcome (live births, fetal deaths, and elective terminations) of any gestational age where the delivery occurred in Hawaii and a reportable birth defect, neoplasm, congenital infection, or prenatal illicit drug use was identified between conception and 1 yr after delivery. Trained HBDP staff collected information on eligible subjects through review of medical records at all delivery and tertiary care pediatric hospitals, facilities that perform elective terminations secondary to prenatal diagnosis of birth defects, genetic counseling centers, cytogenetic laboratories, and all but one of the prenatal ultrasound facilities in Hawaii. Through this multiple source system, ascertainment of infants and fetuses diagnosed with eligible conditions (at least for birth defects, neoplasm, and congenital infections) is believed to be as complete as possible because an eligible infant or fetus missed through one ascertainment source is likely to be identified through another. However, independent verification of this assertion has not been documented.

In order to select which medical records to review, the HBDP provides each health care facility with a list of International Classification of Diseases Ninth Revision (ICD-9) codes that designate conditions of interest to the HBDP. Included on this list are the ICD-9 codes for birth defects (mainly 740–759.9) and for noxious influences affecting the fetus via the placenta or breast milk (760.70–760.79). The first range of codes was used to identify infants and fetuses with birth defects, while the latter range of codes was used to identify illicit drug use during pregnancy.

A diagnosis of illicit drug use during pregnancy was based on any mention of illicit drug use during pregnancy in the medical record or a positive toxicology screen for the mother or infant during or shortly after delivery. In the HBDP database, for verification of illicit drug use a positive toxicology screen is considered to be superior to mention in the medical record. So if an illicit drug has a positive toxicology screen and is mentioned in the medical record, the HBDP database only notes that there was a positive toxicology screen. As a result, there is no way to distinguish those instances where the illicit drug use was based on both methods from those instances where the drug use was based solely on a positive toxicology screen.

Cases for the current investigation consisted of all HBDP infants and fetuses delivered during 1986–2002 with a report of prenatal illicit drug use involving methamphetamine, cocaine, or marijuana or a diagnosis of any of 54 selected birth defects. The three illicit drugs were chosen because they were the drugs most commonly reported in prenatal illicit drug use in Hawaii. The particular birth defects were chosen because they were (1) relatively common defects, (2) easy to diagnose, and/or (3) were associated with increased morbidity or mortality. These 54 birth defects are listed in Tables 1–3. All pregnancy outcomes (live births, fetal deaths, elective terminations) were included because in Hawaii a large proportion of fetuses identified with certain types of birth defects do not result in live birth (Forrester & Merz, 2004; Forrester et al., 1998).

The rate of prenatal use of methamphetamine, cocaine, and marijuana was calculated among the population using the number of live births reported to the Hawaii Department of Health as a denominator. Fetal deaths and elective terminations were not included in the denominators because it is not believed that such pregnancy outcomes are accurately reported to the Department of Health.

The rate of each of the 3 illicit drugs was then calculated for each of the 54 selected birth defects. A portion of mothers used two or more of the illicit drugs investigated during a given pregnancy. These mothers were included in all of the relevant analyses. For example, if the mother used methamphetamine and cocaine, the mother was included in the analysis of methamphetamine and the analysis of cocaine. However, in an effort to minimize confounding by associated illicit drugs, the analyses were also performed using those cases where only one of the illicit drugs was reported to have been used.

TABLE 1

Rate of Prenatal Methamphetamine Use Among Infants and Fetuses With Selected Birth Defects, Hawaii, 1986–2002

Birth defect	Total cases	Total use[a]	Rate (%)	Rate ratio[b]	95% CI[c]	Isolated use[a]	Rate (%)	Rate ratio[b]	95% CI[c]
Anencephaly	118	1	0.85	1.64	0.04–9.29	1	0.85	2.16	0.05–12.28
Spina bifida	144	0	0.00	0.00	0.00–5.01	0	0.00	0.00	0.00–6.62
Encephalocele	63	1	1.59	3.06	0.08–17.70	1	1.59	4.05	0.10–23.39
Holoprosencephaly	38	2	5.26	10.16	1.19–39.30	1	2.63	6.71	0.17–39.72
Hydrocephaly	353	5	1.42	2.73	0.88–6.44	4	1.13	2.89	0.78–7.47
Microcephaly	328	16	4.88	9.41	5.32–16.52	14	4.27	10.89	5.88–18.53
Anophthalmia/microphthalmia	101	6	5.94	11.46	4.11–25.83	3	2.97	7.58	1.54–22.78
Cataract	39	0	0.00	0.00	0.00–19.15	0	0.00	0.00	0.00–25.30
Glaucoma	11	0	0.00	0.00	0.00–76.90	0	0.00	0.00	0.00–101.62
Anotia/microtia	120	3	2.50	4.82	0.98–14.44	3	2.50	6.38	1.30–19.09
Truncus arteriosus	21	0	0.00	0.00	0.00–37.06	0	0.00	0.00	0.00–48.98
Transposition of great arteries	136	4	2.94	5.68	1.53–14.87	4	2.94	7.50	2.01–19.65
Tetralogy of Fallot	123	3	2.44	4.71	0.96–14.08	2	1.63	4.15	0.50–15.30
Single ventricle	28	2	7.14	13.79	1.59–54.67	2	7.14	18.22	2.10–72.24
Ventricular septal defect	1331	27	2.03	3.91	2.57–5.72	16	1.20	3.07	1.75–5.00
Atrial septal defect	686	16	2.33	4.50	2.56–7.36	10	1.46	3.72	1.78–6.88
Endocardial cushion defect	74	2	2.70	5.22	0.62–19.52	2	2.70	6.89	0.82–25.80
Pulmonary valve atresia/stenosis	293	3	1.02	1.98	0.41–5.83	2	0.68	1.74	0.21–6.35
Tricuspid valve atresia/stenosis	53	2	3.77	7.28	0.86–27.64	1	1.89	4.81	0.12–28.00
Ebstein's anomaly	16	1	6.25	12.06	0.29–77.64	1	6.25	15.94	0.38–102.61
Aortic valve stenosis	38	1	2.63	5.08	0.13–30.06	1	2.63	6.71	0.17–39.72
Hypoplastic left heart syndrome	52	0	0.00	0.00	0.00–14.19	0	0.00	0.00	0.00–18.75
Coarctation of aorta		0	0.00	0.00	0.00–9.73	0	0.00	0.00	0.00–12.86
Interrupted aortic arch	14	0	0.00	0.00	0.00–58.18	0	0.00	0.00	0.00–76.89
Anomalous pulmonary venous return	43	0	0.00	0.00	0.00–17.29	0	0.00	0.00	0.00–22.85
Choanal atresia/stenosis	39	0	0.00	0.00	0.00–19.15	0	0.00	0.00	0.00–25.30
Cleft palate	228	8	3.51	6.77	2.89–13.57	6	2.63	6.71	2.44–14.84
Cleft lip with/without cleft palate	410	10	2.44	4.71	2.24–8.75	5	1.22	3.11	1.01–7.32
Esophageal atresia or tracheoesophageal fistula	69	1	1.45	2.80	0.07–16.11	1	1.45	3.70	0.09–21.29
Pyloric stenosis	255	4	1.57	3.03	0.82–7.85	2	0.78	2.00	0.24–7.30
Small-intestinal atresia/stenosis	89	3	3.37	6.51	1.32–19.64	2	2.25	5.73	0.68–21.32
Anal, rectal, and large-intestinal atresia/stenosis	162	3	1.85	3.57	0.73–10.63	2	1.23	3.15	0.38–11.56

(*Continued*)

TABLE 1
(*Continued*)

Birth defect	Total cases	Total use[a]	Rate (%)	Rate ratio[b]	95% CI[c]	Isolated use[a]	Rate (%)	Rate ratio[b]	95% CI[c]
Hirschsprung's disease	69	0	0.00	0.00	0.00–10.60	0	0.00	0.00	0.00–14.01
Biliary atresia	34	0	0.00	0.00	0.00–22.12	0	0.00	0.00	0.00–29.23
Malrotation of intestines	91	0	0.00	0.00	0.00–7.98	0	0.00	0.00	0.00–10.55
Hypospadias and epispadias	856	6	0.70	1.35	0.50–2.96	5	0.58	1.49	0.48–3.49
Renal agenesis or hypoplasia	146	1	0.68	1.32	0.03–7.48	0	0.00	0.00	0.00–6.53
Cystic kidney	144	1	0.69	1.34	0.03–7.59	1	0.69	1.77	0.05–10.03
Obstructive genitourinary defect	455	4	0.88	1.70	0.46–4.37	3	0.66	1.68	0.35–4.95
Bladder exstrophy	9	0	0.00	0.00	0.00–97.78	0	0.00	0.00	0.00–129.21
Persistent cloaca	5	0	0.00	0.00	0.00–210.61	0	0.00	0.00	0.00–278.32
Congenital hip dislocation	312	3	0.96	1.86	0.38–5.47	3	0.96	2.45	0.50–7.23
Polydactyly	568	11	1.94	3.74	1.86–6.74	9	1.58	4.04	1.84–7.73
Syndactyly	276	7	2.54	4.89	1.95–10.22	4	1.45	3.70	1.00–9.57
Reduction deformity of upper limbs	115	3	2.61	5.03	1.02–15.09	0	0.00	0.00	0.00–8.31
Reduction deformity of lower limbs	47	2	4.26	8.21	0.97–31.36	0	0.00	0.00	0.00–20.82
Craniosynostosis	159	0	0.00	0.00	0.00–4.53	0	0.00	0.00	0.00–5.99
Diaphragmatic hernia	78	1	1.28	2.47	0.06–14.20	0	0.00	0.00	0.00–12.35
Omphalocele	90	1	1.11	2.14	0.05–12.26	1	1.11	2.83	0.07–16.20
Gastroschisis	109	1	0.92	1.77	0.04–10.07	1	0.92	2.34	0.06–13.31
Situs inversus	35	2	5.71	11.03	1.29–42.93	2	5.71	14.57	1.70–56.73
Trisomy 21	479	6	1.25	2.42	0.88–5.30	6	1.25	3.19	1.17–7.01
Trisomy 13	62	0	0.00	0.00	0.00–11.83	0	0.00	0.00	0.00–15.64
Trisomy 18	152	1	0.66	1.27	0.03–7.18	1	0.66	1.68	0.04–9.49
Total live births	316,508	1640	0.52	ref		1241	0.39	ref	

Note. A delivery with more than one structural birth defect will be included in all relevant categories.
[a]Total use = all cases of methamphetamine use. Isolated use = cases of methamphetamine use excluding those cases where cocaine or marijuana were also used.
[b]Ratio of the rate of illicit drug use among birth defect cases to the rate of illicit drug use among all deliveries.
[c]CI = confidence interval.

TABLE 2
Rate of Prenatal Cocaine Use Among Infants and Fetuses With Selected Birth Defects, Hawaii, 1986–2002

Birth defect	Total cases	Total use[a]	Rate (%)	Rate ratio[b]	95% CI[c]	Isolated use[a]	Rate (%)	Rate ratio[b]	95% CI[c]
Anencephaly	118	0	0.00	0.00	0.00–17.85	0	0.00	0.00	0.00–30.64
Spina bifida	144	0	0.00	0.00	0.00–14.59	0	0.00	0.00	0.00–25.04
Encephalocele	63	0	0.00	0.00	0.00–33.90	0	0.00	0.00	0.00–58.19
Holoprosencephaly	38	0	0.00	0.00	0.00–57.31	0	0.00	0.00	0.00–98.37
Hydrocephaly	353	4	1.13	6.37	1.73–16.46	2	0.57	5.47	0.66–19.90
Microcephaly	328	2	0.61	3.43	0.41–12.48	1	0.30	2.94	0.07–16.51
Anophthalmia/microphthalmia	101	2	1.98	11.13	1.33–41.27	1	0.99	9.55	0.24–54.45
Cataract	39	1	2.56	14.41	0.36–85.18	1	2.56	24.74	0.61–146.22
Glaucoma	11	0	0.00	0.00	0.00–223.99	0	0.00	0.00	0.00–384.47
Anotia/microtia	120	0	0.00	0.00	0.00–17.55	0	0.00	0.00	0.00–30.12
Truncus arteriosus	21	0	0.00	0.00	0.00–107.96	0	0.00	0.00	0.00–185.31
Transposition of great arteries	136	2	1.47	8.27	0.99–30.44	2	1.47	14.19	1.70–52.26
Tetralogy of Fallot	123	3	2.44	13.71	2.79–41.02	1	0.81	7.85	0.20–44.53
Single ventricle	28	0	0.00	0.00	0.00–79.17	0	0.00	0.00	0.00–135.88
Ventricular septal defect	1331	20	1.50	8.45	5.14–13.10	14	1.05	10.15	5.53–17.09
Atrial septal defect	686	9	1.31	7.38	3.36–14.08	5	0.73	7.03	2.28–16.49
Endocardial cushion defect	74	0	0.00	0.00	0.00–28.74	0	0.00	0.00	0.00–49.32
Pulmonary valve atresia/stenosis	293	5	1.71	9.59	3.09–22.64	5	1.71	16.47	5.31–38.87
Tricuspid valve atresia/stenosis	53	1	1.89	10.61	0.26–61.71	1	1.89	18.21	0.45–105.93
Ebstein's anomaly	16	0	0.00	0.00	0.00–145.77	0	0.00	0.00	0.00–250.21
Aortic valve stenosis	38	0	0.00	0.00	0.00–57.31	0	0.00	0.00	0.00–98.37
Hypoplastic left heart syndrome	52	0	0.00	0.00	0.00–41.33	0	0.00	0.00	0.00–70394
Coarctation of aorta	75	2	2.67	14.99	1.78–56.07	2	2.67	25.73	3.06–96.25
Interrupted aortic arch	14	0	0.00	0.00	0.00–169.48	0	0.00	0.00	0.00–290.90
Anomalous pulmonary venous return	43	0	0.00	0.00	0.00–50.36	0	0.00	0.00	0.00–86.44
Choanal atresia/stenosis	39	0	0.00	0.00	0.00–55.77	0	0.00	0.00	0.00–95.73
Cleft palate	228	2	0.88	4.93	0.59–18.02	2	0.88	8.46	1.02–30.93
Cleft lip with/without cleft palate	410	6	1.46	8.23	3.00–18.06	2	0.49	4.71	0.57–17.11
Esophageal atresia or tracheoesophageal fistula	69	1	1.45	8.15	0.20–46.93	1	1.45	13.98	0.35–80.55
Pyloric stenosis	255	3	1.18	6.61	1.36–19.55	1	0.39	3.78	0.10–21.27

(*Continued*)

TABLE 2
(*Continued*)

Birth defect	Total cases	Total use[a]	Rate (%)	Rate ratio[b]	95% CI[c]	Isolated use[a]	Rate (%)	Rate ratio[b]	95% CI[c]
Small-intestinal atresia/stenosis	89	1	1.12	6.32	0.16–36.11	1	1.12	10.84	0.27–61.98
Anal, rectal, and large-intestinal atresia/stenosis	162	1	0.62	3.47	0.09–19.61	1	0.62	5.96	0.15–33.66
Hirschsprung's disease	69	0	0.00	0.00	0.00–30.87	0	0.00	0.00	0.00–52.99
Biliary atresia	34	1	2.94	16.53	0.41–98.57	1	2.94	28.38	0.70–169.18
Malrotation of intestines	91	0	0.00	0.00	0.00–23.26	0	0.00	0.00	0.00–39.92
Hypospadias and epispadias	856	1	0.12	0.66	0.02–3.67	1	0.12	1.13	0.03–6.30
Renal agenesis or hypoplasia	146	1	0.68	3.85	0.10–21.79	1	0.68	6.61	0.17–37.41
Cystic kidney	144	2	1.39	7.81	0.94–28.72	2	1.39	13.40	1.61–49.30
Obstructive genitourinary defect	455	4	0.88	4.94	1.34–12.74	3	0.66	6.36	1.30–18.71
Bladder exstrophy	9	0	0.00	0.00	0.00–284.82	0	0.00	0.00	0.00–488.88
Persistent cloaca	5	0	0.00	0.00	0.00–613.50	0	0.00	0.00	0.00–1053.04
Congenital hip dislocation	312	2	0.64	3.60	0.44–13.13	1	0.32	3.09	0.08–17.36
Polydactyly	568	5	0.88	4.95	1.60–11.62	5	0.88	8.49	2.75–19.94
Syndactyly	276	5	1.81	10.18	3.28–24.06	3	1.09	10.49	2.15–30.97
Reduction deformity of upper limbs	115	4	3.48	19.55	5.24–51.44	3	2.61	25.17	5.12–75.43
Reduction deformity of lower limbs	47	1	2.13	11.96	0.30–69.98	0	0.00	0.00	0.00–78.79
Craniosynostosis	159	0	0.00	0.00	0.00–13.20	0	0.00	0.00	0.00–22.65
Diaphragmatic hernia	78	1	1.28	7.21	0.18–41.35	0	0.00	0.00	0.00–46.73
Omphalocele	90	1	1.11	6.25	0.16–35.70	0	0.00	0.00	0.00–40.37
Gastroschisis	109	1	0.92	5.16	0.13–29.35	1	0.92	8.85	0.22–50.37
Situs inversus	35	0	0.00	0.00	0.00–62.49	0	0.00	0.00	0.00–107.26
Trisomy 21	479	0	0.00	0.00	0.00–4.35	0	0.00	0.00	0.00–7.46
Trisomy 13	62	1	1.61	9.07	0.23–52.42	1	1.61	15.56	0.39–89.98
Trisomy 18	152	0	0.00	0.00	0.00–13.81	0	0.00	0.00	0.00–23.71
Total live births	316,508	563	0.18	ref		328	0.10	ref	

Note. A delivery with more than one structural birth defect will be included in all relevant categories.
[a]Total use = all cases of cocaine use. Isolated use = cases of cocaine use excluding those cases where methamphetamine or marijuana were also used.
[b]Ratio of the rate of illicit drug use among birth defect cases to the rate of illicit drug use among all deliveries.
[c]CI = confidence interval.

TABLE 3
Rate of Prenatal Marijuana Use Among Infants and Fetuses With Selected Birth Defects, Hawaii, 1986–2002

Birth defect	Total cases	Total use[a]	Rate (%)	Rate ratio[b]	95% CI[c]	Isolated use[a]	Rate (%)	Rate ratio[b]	95% CI[c]
Anencephaly	118	0	0.00	0.00	0.00–12.14	0	0.00	0.00	0.00–26.66
Spina bifida	144	0	0.00	0.00	0.00–12.57	0	0.00	0.00	0.00–21.79
Encephalocele	63	3	4.76	18.20	3.66–55.68	3	4.76	39.98	8.03–122.29
Holoprosencephaly	38	2	5.26	20.12	2.35–77.85	1	2.63	22.09	0.55–130.76
Hydrocephaly	353	8	2.27	8.66	3.71–17.26	7	1.98	16.65	6.65–34.66
Microcephaly	328	8	2.44	9.32	3.99–18.59	5	1.52	12.80	4.13–30.17
Anophthalmia/microphthalmia	101	3	2.97	11.35	2.30–34.14	1	0.99	8.31	0.21–47.38
Cataract	39	0	0.00	0.00	0.00–37.92	0	0.00	0.00	0.00–83.29
Glaucoma	11	0	0.00	0.00	0.00–152.30	0	0.00	0.00	0.00–334.50
Anotia/microtia	120	2	1.67	6.37	0.76–23.52	2	1.67	13.99	1.68–51.66
Truncus arteriosus	21	0	0.00	0.00	0.00–73.41	0	0.00	0.00	0.00–161.22
Transposition of great arteries	136	1	0.74	2.81	0.07–15.93	1	0.74	6.17	0.16–34.98
Tetralogy of Fallot	123	3	2.44	9.32	1.90–27.89	2	1.63	13.65	1.64–50.37
Single ventricle	28	0	0.00	0.00	0.00–53.83	0	0.00	0.00	0.00–118.22
Ventricular septal defect	1331	25	1.88	7.18	4.63–10.65	14	1.05	8.83	4.82–14.87
Atrial septal defect	686	12	1.75	6.69	3.44–11.76	5	0.73	6.12	1.98–14.35
Endocardial cushion defect	74	0	0.00	0.00	0.00–19.54	0	0.00	0.00	0.00–42.91
Pulmonary valve atresia/stenosis	293	5	1.71	6.52	2.10–16.40	4	1.37	11.46	3.10–29.66
Tricuspid valve atresia/stenosis	53	1	1.89	7.21	0.18–41.96	0	0.00	0.00	0.00–60.52
Ebstein's anomaly	16	0	0.00	0.00	0.00–99.12	0	0.00	0.00	0.00–217.69
Aortic valve stenosis	38	1	2.63	10.06	0.25–59.54	1	2.63	22.09	0.55–130.76
Hypoplastic left heart syndrome	52	2	3.85	14.70	1.74–55.85	2	3.85	32.29	3.81–122.65
Coarctation of aorta	75	1	1.33	5.10	0.13–29.28	1	1.33	11.19	0.28–64.30
Interrupted aortic arch	14	0	0.00	0.00	0.00–115.24	0	0.00	0.00	0.00–253.09
Anomalous pulmonary venous return	43	0	0.00	0.00	0.00–34.24	0	0.00	0.00	0.00–75.20
Choanal atresia/stenosis	39	0	0.00	0.00	0.00–37.92	0	0.00	0.00	0.00–83.29
Cleft palate	228	6	2.63	10.06	3.65–22.24	4	1.75	14.73	3.98–38.23
Cleft lip with/without cleft palate	410	7	1.71	6.53	2.61–13.57	4	0.98	8.19	2.22–21.13
Esophageal atresia or tracheoesophageal fistula	69	0	0.00	0.00	0.00–20.99	0	0.00	0.00	0.00–46.11
Pyloric stenosis	255	5	1.96	7.50	2.41–17.72	4	1.57	13.17	3.56–34.13
Small-intestinal atresia/stenosis	89	2	2.25	8.59	1.02–31.95	1	1.12	9.43	0.24–53.93
Anal, rectal, and large-intestinal atresia/stenosis	162	3	1.85	7.08	1.46–21.06	2	1.23	10.36	1.25–38.05
Hirschsprung's disease	69	0	0.00	0.00	0.00–20.99	0	0.00	0.00	0.00–46.11
Biliary atresia	34	0	0.00	0.00	0.00–43.81	0	0.00	0.00	0.00–96.21
Malrotation of intestines	91	1	1.10	4.20	0.11–24.00	1	1.10	9.23	0.23–52.71

(*Continued*)

TABLE 3
(*Continued*)

Birth defect	Total cases	Total use[a]	Rate (%)	Rate ratio[b]	95% CI[c]	Isolated use[a]	Rate (%)	Rate ratio[b]	95% CI[c]
Hypospadias and epispadias	856	4	0.47	1.79	0.49–4.59	3	0.35	2.94	0.61–8.63
Renal agenesis or hypoplasia	146	2	1.37	5.24	0.63–19.26	1	0.68	5.75	0.15–32.55
Cystic kidney	144	1	0.69	2.65	0.07–15.03	1	0.69	5.83	0.15–33.00
Obstructive genitourinary defect	455	7	1.54	5.88	2.35–12.22	5	1.10	9.23	2.98–21.69
Bladder exstrophy	9	0	0.00	0.00	0.00–193.66	0	0.00	0.00	0.00–425.34
Persistent cloaca	5	0	0.00	0.00	0.00–417.15	0	0.00	0.00	0.00–916.18
Congenital hip dislocation	312	1	0.32	1.23	0.03–6.88	0	0.00	0.00	0.00–9.99
Polydactyly	568	8	1.41	5.38	2.31–10.68	6	1.06	8.87	3.24–19.42
Syndactyly	276	13	4.71	18.00	9.47–31.30	8	2.90	24.33	10.40–48.63
Reduction deformity of upper limbs	115	7	6.09	23.27	9.15–49.50	3	2.61	21.90	4.45–65.63
Reduction deformity of lower limbs	47	3	6.38	24.40	4.86–75.80	0	0.00	0.00	0.00–68.55
Craniosynostosis	159	0	0.00	0.00	0.00–8.97	0	0.00	0.00	0.00–19.71
Diaphragmatic hernia	78	0	0.00	0.00	0.00–18.51	0	0.00	0.00	0.00–40.66
Omphalocele	90	1	1.11	4.25	0.11–24.27	0	0.00	0.00	0.00–35.13
Gastroschisis	109	3	2.75	10.52	2.14–31.57	3	2.75	23.11	4.69–69.34
Situs inversus	35	1	2.86	10.92	0.27–64.98	1	2.86	23.99	0.59–142.71
Trisomy 21	479	3	0.63	2.39	0.49–7.04	3	0.63	5.26	1.08–15.46
Trisomy 13	62	0	0.00	0.00	0.00–23.43	0	0.00	0.00	0.00–51.47
Trisomy 18	152	0	0.00	0.00	0.00–9.39	0	0.00	0.00	0.00–20.62
Total live births	316,508	828	0.26	ref		377	0.12	ref	

Note. A delivery with more than one structural birth defect will be included in all relevant categories.
[a]Total use = all cases of marijuana use. Isolated use = cases of marijuana use excluding those cases where methamphetamine or cocaine were also used.
[b]Ratio of the rate of illicit drug use among birth defect cases to the rate of illicit drug use among all deliveries.
[c]CI = confidence interval.

The illicit drug use rates among the birth defects were then compared to the rate among all births by calculating the rate ratio and 95% confidence interval (CI) using Poisson probability.

RESULTS

The HBDP identified 1640 cases of prenatal methamphetamine use, 563 cases of prenatal cocaine use, and 829 cases of prenatal marijuana use among deliveries during 1986–2002. During the same time period, there were 316,508 live births reported in Hawaii. Thus the prenatal use rate was 0.52% for methamphetamine, 0.18% for cocaine, and 0.26% for marijuana. If cases where 2 or more of the illicit drugs were used are excluded, there were 1241 cases of prenatal methamphetamine use, 328 cases of prenatal cocaine use, and 377 cases of prenatal marijuana use. The prenatal use rates for isolated exposures were then 0.39% for methamphetamine, 0.10% for cocaine, and 0.12% for marijuana.

During this 17-yr time period, there were 7293 infants and fetuses with one or more of the 54 birth defects of interest. Of these cases, 6545 (89.7%) were live births, 207 (2.8%) fetal deaths, 527 (7.2%) elective terminations, and 14 (0.2%) unknown pregnancy outcome. The live birth rate varied from 16.1% for anencephaly to 100% for cataract, glaucoma, interrupted aortic arch, choanal atresia/stenosis, Hirschsprung's disease, persistent cloaca, and craniosynostosis.

Table 1 contains the prenatal methamphetamine use rate among selected birth defects. Prenatal methamphetamine rates were significantly higher than expected for 14 (26%) of the birth defects. Most of these defects involved the central nervous system (holoprosencephaly, microcephaly), cardiovascular system (transposition of great arteries, single ventricle, ventricular septal defect, atrial septal defect), oral clefts (cleft palate alone, cleft lip with/without cleft palate), and limbs (polydactyly, syndactyly, reduction deformity of upper limbs). Other birth defects with significantly higher than expected prenatal methamphetamine rates were anophthalmia/microphthalmia, small-intestinal atresia/stenosis, and situs inversus. If the analysis was restricted only to those cases where methamphetamine alone was used, then the rates were significantly higher than expected for 12 (22%) of the birth defects (microcephaly, anophthalmia/microphthalmia, anotia/microtia, transposition of great arteries, single ventricle, ventricular septal defect, atrial septal defect, cleft palate alone, cleft lip with/without cleft palate, polydactyly, situs inversus, trisomy 21).

Table 2 presents the prenatal cocaine use rate for the same birth defects. Prenatal cocaine rates were significantly higher than expected for 13 (24%) of the birth defects. These defects were primarily associated with the central nervous system (hydrocephaly), cardiovascular system (tetralogy of Fallot, ventricular septal defect, atrial septal defect, pulmonary valve atresia/stenosis, coarctation of aorta), oral clefts (cleft lip with/without cleft palate), and limbs (polydactyly, syndactyly, reduction deformity of upper limbs). Other birth defects with significantly higher than expected cocaine rates were anophthalmia/microphthalmia, pyloric stenosis, and obstructive genitourinary defect. If the analysis included only the cases where cocaine alone was reported, then the rates were significantly higher than expected for 11 (20%) of the birth defects (transposition of great arteries, ventricular septal defect, atrial septal defect, pulmonary valve atresia/stenosis, coarctation of aorta, cleft palate alone, cystic kidney, obstructive genitourinary defect, polydactyly, syndactyly, reduction deformity of upper limbs).

Table 3 shows the prenatal marijuana use rate for the 54 birth defects. Prenatal marijuana rates were significantly higher than expected for 21 (39%) of the birth defects. The birth defects with greater than expected prenatal marijuana use rates were mainly defects of the central nervous system (encephalocele, holoprosencephaly, hydrocephaly, microcephaly), cardiovascular system (tetralogy of Fallot, ventricular septal defect, atrial septal defect, pulmonary valve atresia/stenosis, hypoplastic left heart syndrome), oral clefts (cleft palate alone, cleft lip with/without cleft palate), gastrointestinal system (pyloric stenosis, small-intestinal atresia/stenosis, anal/rectal/large-intestinal atresia/stenosis), and limbs (polydactyly, syndactyly, reduction deformity of upper limbs, reduction deformity of lower limbs). Other birth defects with significantly increased prenatal marijuana rates were anophthalmia/microphthalmia, obstructive genitourinary defect, and gastroschisis. If the analysis was limited to those cases where marijuana by itself was used, then the rates were significantly higher than expected for 19 (35%) of the birth defects (encephalocele, hydrocephaly, microcephaly, anotia/microtia, tetralogy of Fallot, ventricular septal defect, atrial septal defect, pulmonary valve atresia/stenosis, hypoplastic left heart syndrome, cleft palate alone, cleft lip with/without cleft palate, pyloric stenosis, anal/rectal/large-intestinal atresia/stenosis, obstructive genitourinary defect, polydactyly, syndactyly, reduction deformity of upper limbs, gastroschisis, trisomy 21).

DISCUSSION

Using data from a statewide, population-based registry that covered over 300,000 births and a 17-yr period, this investigation examined the association between over 50 selected birth defects and maternal use of methamphetamine, cocaine, or marijuana during pregnancy. Much of the literature on prenatal illicit drug use and birth defects involved case reports, involved a small number of cases, were not population-based, or focused on only one or a few particular birth defects.

There are various limitations to this investigation. The number of cases for many of the birth defects categories was relatively small, limiting the ability to identify statistically significant differences and resulting in large confidence intervals. In spite of this, a number of statistically significant analyses were identified. Some statistically significant results might

be expected to occur by chance. If 1 in every 20 analyses is expected to result in statistically significant differences solely by chance, then among the 162 analyses performed in this study, 8 would be expected to be statistically significant by chance. However, 48 statistically significant differences were identified. Thus, not all of the statistically significant results are likely to be due to chance.

This study included all pregnancies where methamphetamine, cocaine, or marijuana use was identified through either report in the medical record or positive toxicology test. This was done because neither self-report nor toxicology testing is likely to identify all instances of prenatal illicit drug use (Christmas et al., 1992). In spite of using both methods for determining prenatal illicit drug use, all pregnancies involving methamphetamine, cocaine, or marijuana were not likely to have been identified. The degree of under ascertainment is unknown. A previous study examined the maternal drug use rate around the time of delivery in Hawaii during 1999 (Derauf et al., 2003). This study found 1.4% of the pregnancies involved methamphetamine use and 0.2% involved marijuana use. Among 1999 deliveries, the HBDP identified a prenatal methamphetamine use rate of 0.7% and a marijuana use rate of 0.4%. However, comparisons between the 2 studies should be made with caution because the previous study collected data from a single hospital during only a 2-mo period.

Another limitation is that the present study did not control for potential confounding factors such as maternal demographic characteristics, health behaviors, and prenatal care. Increased risk of birth defects has been associated with inadequate prenatal care (Carmichael et al., 2002), maternal smoking (Honein et al., 2001), and maternal alcohol use (Martinez-Frias et al., 2004). These factors are also found with maternal illicit drug use (Cosden et al., 1997; Hutchins, 1997; Norton-Hawk, 1997). Thus the increased risk of selected birth defects with illicit drug use in this study might actually be due to one of these other underlying factors. Unfortunately, information on some of the potential confounding factors such as socioeconomic status are not collected by the HBDP. Information collected on some other factors such as smoking and alcohol use is suspect because of negative attitudes toward their use during pregnancy. Moreover, the small number of cases among many of the birth defects groups would make controlling for these factors difficult.

Finally, this investigation included use of the illicit drugs at any time during the pregnancy. Most birth defects are believed to occur at 3–8 wk after conception (Makri et al., 2004; Sadler, 2000). In a portion of the cases, the drug use might have occurred at a time when it could not have caused the birth defect. Furthermore, this study does not include information on dose; however, teratogenicity of a substance may depend on its dose (Werler et al., 1990). In spite of the various potential concerns of the present study, data may suggest future areas of investigation where the limitations inherent in the present one are excluded.

This investigation found significantly higher than expected rates for prenatal use of methamphetamine, cocaine, and marijuana among a number of specific birth defects. Although not identical, there were general similarities between the three illicit drugs and the birth defects with which they were associated. Increased rates for methamphetamine, cocaine, and marijuana occurred predominantly among birth defects affecting the central nervous system, cardiovascular system, oral clefts, and limbs. There were also increased rates of marijuana use with a variety of birth defects associated with the gastrointestinal system. With the exception of marijuana and encephalocele, none of illicit drugs were associated with neural-tube defects (anencephaly, spina bifida, encephalocele). The rates of use for the three illicit drugs were not significantly elevated with eye defects other than anophthalmia/microphthalmia, genitourinary defects, and musculoskeletal defects aside from limb defects. In the majority of instances, the associations between particular illicit drugs and birth defects were found whether or not those cases involving use of multiple types of drugs were included. Of the 14 significant associations between methamphetamine and specific birth defects, 10 (71.4%) remained once multiple drug cases were excluded. Corresponding rates were 61.5% (8 of 13) for cocaine and 81.0% (17 of 21) for marijuana.

The similarities in the patterns of birth defects with which methamphetamine, cocaine, and marijuana are associated might suggest that the three drugs exert similar effects on embryonic and fetal development. This might not be expected, considering that the three illicit drugs differ in their mechanisms of action and clinical effects (Leiken & Paloucek, 1998).

Some of the associations between methamphetamine, cocaine, and marijuana observed in the present investigation were previously reported. Other studies observed similar associations, or lack thereof, of methamphetamine or amphetamine with neural-tube defects (Shaw et al., 1996) and cardiovascular and musculoskeletal defects (McElhatton et al., 2000); cocaine with neural-tube defects (Shaw et al., 1996), cardiovascular defects (Lipshultz et al., 1991), ventricular septal defect and atrial septal defect (Ferencz et al., 1997c; Martin & Edmonds, 1991), tricuspid atresia (Ferencz et al., 1997d), craniosynostosis (Gardner et al., 1998), and situs inversus (Kuehl & Loffredo, 2002); and marijuana with neural-tube defects (Shaw et al., 1996), single ventricle (Steinberger et al., 2002), ventricular septal defect (Williams et al., 2004), tricuspid atresia (Ferencz et al., 1997d), and gastroschisis (Torfs et al., 1994).

In contrast, this study differed from other research with respect to their findings regarding methamphetamine or amphetamine and gastroschisis (Torfs et al., 1994); cocaine and microcephaly (Martin & Edmonds, 1991), conotruncal defects (Adams et al., 1989), endocardial cushion defect (Ferencz et al., 1997b), situs inversus (Ferencz et al., 1997a), oral clefts (Beaty et al., 2001), and genitourinary defects (Abe et al., 2003; Battin et al., 1995; Martin & Edmonds, 1991); and marijuana and conotruncal defects (Adams et al., 1989), Ebstein anomaly (Ferencz et al., 1997e; Correa-Villasenor et al., 1994), and oral

clefts (Beaty et al., 2001). The inconsistent findings between this and the other studies could be due to differences in study methodology, case classification, or number of cases.

The mechanisms by which methamphetamine, cocaine, and marijuana might contribute to the rates for birth defects is currently unknown. Any potential explanation would have to take into account the observation that each of the illicit drugs was associated with a variety of specific birth defects affecting different organ systems. This might suggest that these three drugs would need to influence a basic, common factor involved in embryonic development.

Folic acid is involved in nucleic acid synthesis and cellular division (Scholl & Johnson, 2000) and thus would play an important role in the early growth and cellular proliferation of the embryo. Folic acid has been found to prevent a variety of birth defects (Forrester & Merz, 2005). Thus, anything that interferes with the activity of folic acid might be expected to increase the risk for these birth defects. Many of these birth defects were associated with methamphetamine, cocaine, and/or marijuana in the present study. However, two of the birth defects most closely affected by folic acid—anencephaly and spina bifida—were not associated with any of the three illicit drugs.

Vascular disruption has been suggested as a potential cause for a variety of different birth defects such as intestinal atresia/stenosis, limb reduction defects, and gastroschisis. Since cocaine is a vasoconstrictor, it has been hypothesized that cocaine use could increase the risk of these vascular disruption defects (Hume et al., 1997; Martin et al., 1992; Hoyme et al., 1983; de Vries, 1980). Although this investigation found an association between cocaine and limb reduction deformities, no association was found with intestinal atresia/stenosis or gastroschisis.

In conclusion, this study found that prenatal use of methamphetamine, cocaine, or marijuana were associated with increased risk of a variety of birth defects. The affected birth defects were primarily associated with particular organ systems. Because of various limitations of the study, further research is recommended.

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Summary of Cannabis Genotoxicity Papers

There are several principal pathways to inheritable genotoxicity, mutagenicity and teratogenesis induced by cannabis which are known and well established at this time including the following. These three papers discuss different aspects of these effects.

1) **Stops Brain Waves and Thinking** The brain has both stimulatory and inhibitory pathways. GABA is the main brain inhibitory pathway. Brain centres talk to each other on gamma (about 40 cycles/sec) and theta frequencies (about 5 cycles/sec), where the theta waves are used as the carrier waves for the gamma wave which then interacts like harmonics in music. The degree to which the waves are in and out of phase carries information which can be monitored externally. GABA (γ-aminobutyric acid) inhibition is key to the generation of the synchronized firing which underpins these various brain oscillations. These GABA transmissions are controlled presynaptically by type 1 cannabinoid receptors (CB1R's) and CB1R stimulation shuts them down. This is why cannabis users forget and fall asleep.

2) **Blocks GABA Pathway and Brain Formation** GABA is also a key neurotransmitter in brain formation in that it guides and direct neural stem cell formation and transmission and development and growth of the cerebral cortex and other major brain areas. Gamma and theta brain waves also direct neural stem cell formation, sculpting and connectivity. Derangements then of GABA physiology imply that the brain will not form properly. Thin frontal cortical plate measurements have been shown in humans prenatally exposed to cannabis by fMRI. This implies that their brains can never be structurally normal which then explains the long lasting and persistent defects identified into adulthood.

3) **Epigenetic Damage** DNA not only carries the genetic hardware of our genetic code but it also carries the software of the code which works like traffic lights along the sequence of DNA bases to direct when to switch the genes on and off. This is known as the "epigenetic code". Fetal alcohol syndrome is believed to be due to damage to the software epigenetic code. The long lasting intellectual, mood regulation, attention and concentration defects which have been described after in utero cannabis exposure in the primary, middle and high schools and as college age young adults are likely due to these defects. Epigenetics "sets in stone" the errors of brain structure made in (2) above.

4) **Arterial Damage**. Cannabis has a well described effect to damage arteries through (CB1R's) (American Heart Association 2007) which they carry in high concentration (Nature Reviews Cardiology 2018). In adults this causes heart attack (500% elevation in the first hour after smoking), stroke, severe cardiac arrhythmias including sudden cardiac death; but in developing babies CB1R's acting on the developing heart tissues can lead to at least six major cardiac defects (Atrial- ventricular- and mixed atrio-ventricular and septal defects, Tetralogy of Fallot, Epstein's deformity amongst others), whilst constriction of various babies' arteries can lead to serious side effects such as gastroschisis (bowels hanging out) and possibly absent limbs (in at least one series).

5) **Disruption of Mitotic Spindle**. When cells divide the separating chromosomes actually slide along "train tracks" which are long chains made of tubulin. The tubulin chains are called "microtubules" and the whole football-shaped structure is called a "mitotic spindle". Cannabis inhibits tubulin formation, disrupting microtubules and the mitotic spindle causing the separating chromosomes to become cut off in tiny micronuclei, where they eventually become smashed up and pulverized into "genetic junk", which leads to foetal malformations, cancer and cell death. High rates of Down's syndrome, chromosomal anomalies and cancers in cannabis exposed babies provide clinical evidence of this.

6) **Defective Energy Generation & Downstream DNA Damage** DNA is the crown jewel of the cell and its most complex molecule. Maintaining it in good repair is a very energy

intensive process. Without energy DNA cannot be properly maintained. Cannabis has been known to reduce cellular energy production by the cell's power plants, mitochondria, for many decades now. This has now been firmly linked with increased DNA damage, cancer formation and aging of the cells and indeed the whole organism. As it is known to occur in eggs and sperm, this will also damage the quality of the germ cells which go into forming the baby and lead directly to damaged babies and babies lost and wasted through spontaneous miscarriage and therapeutic termination for severe deformities.

7) **Cancer induction** Cannabis causes 12 cancers and has been identified as a carcinogen by the California Environmental Protection agency (2009). This makes it also a mutagen. 4 of these cancers are inheritable to children; i.e. inheritable carcinogenicity and mutagenicity. All four studies in testicular cancer are strongly positive (elevation by three fold). Carcinogen = mutagen = teratogen.

8) **Colorado's Teratology Profile**. From the above described teratological profile we would expect exactly the profile of congenital defects which have been identified in Colorado (higher total defects and heart defects, and chromosomal defects) and Ottawa in Canada (long lasting and persistent brain damage seen on both functional testing and fMRI brain scans in children exposed in utero) where cannabis use has become common. Gastroschisis was shown to be higher in all seven studies looking at this; and including in Canada, carefully controlled studies. Moreover in Australia, Canada, North Carolina, Colorado, Mexico and New Zealand, gastroschisis and sometimes other major congenital defects cluster where cannabis use is highest. Colorado 2000-2013 has experienced an extra 20,152 severely abnormal births above the rates prior to cannabis liberalization which if applied to the whole USA would equate to more than 83,000 abnormal babies live born annually (and probably about that number again therapeutically aborted); actually much more since both the number of users and concentration of cannabis have risen sharply since 2013, and cannabis has been well proven to be much more severely genotoxic at higher doses.

9) **Cannabidiol is also Genotoxic** and tests positive in many genotoxicity assays, just as tetrahydrocannabinol does.

10) **Births defects registry data needs to be open and transparent and public.** At present it is not. This looks too much like a cover up.

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15th April 2018

Dockets Management Staff (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Rm. 1061
Rockville, MD 20852

Re: Department of Health and Human Services, Food and Drug Administration [Docket No. FDA-2018-N-1072]: International Drug Scheduling; Convention on Psychotropic Substances; Single Convention on Narcotic Drugs; Cannabis Plant and Resin; Extracts and Tinctures of Cannabis; Delta-9-Tetrahydrocannabinol; Stereoisomers of Tetrahydrocannabinol; Cannabidiol; **Request for Comments** (FR Doc. 2018-07225).

Federal Register Submission

Re: ***Re-Scheduling of Cannabinoids in USA***
Pattern of Colorado Birth Defects 2000-2013

As a researcher I am concerned about the public health impacts of the known genotoxic effects of cannabis at the population health level.

One of the more obvious places to look to pick up clues that this might be acting is in the Registers of Birth Defects. Unfortunately it appears that extracting quantitative data on birth defects is very difficult as very few make their data publicly available. I have written to Hawaii, Colorado, California, CDC Atlanta, Georgia and MACDP Atlanta, Georgia but as at the time of writing have not had meaningful responses.

Naturally your office is in a much better position to request data urgently from your counterparts in other branches of the American Government and I would strongly urge you to do so.

However a friend was able to send me a link to a registry in Colorado which is of some use and more than a little interest. The data is so concerning that I wished to bring it to your attention. The following notes are written as a commentary on the attached short slide series. Note that the data from the Colorado Registry is supplied only by a single abnormality one at a time, and only for a single year, one at a time. Hence actually downloading the data is very time consuming and more than a little laborious. The two URL's concerned to the Colorado Health Information Dataset are http://www.chd.dphe.state.co.us/cohid/ and http://www.cohid.dphe.state.co.us/scripts/htmsql.exe/CrcsnPub.hsql . Colorado legalized cannabis for recreational use in 2012 and then again fully for recreational use in 2014. Hence the 2014 births defects data is of particular interest. I am told that this data was to be released four months ago, but at the time of writing it is not available.

The data series achieves particular significance in the light of a previously cited teratological literature linking cannabis to various major congenital malformations.

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It should be noted that a major factor in interpreting these curves is the termination rate. Since therapeutic termination is a major management option chosen by many parents for the more severe defects, and widely recommended by many obstetricians, one cannot really form a comprehensive understanding of the applicable trends without knowledge of and due consideration to, the associated antenatal termination rate for the applicable defect.

Both for this reason, and because the data only goes to 2013 it is considered that this data is only reflecting the lower bound of the effects in question. That is to say that these estimates form a lower estimate of the putative cannabis -related teratogenic effect.

Slide Series

Slide 1 (S1) introduces a title slide for this slide series.

S2 shows the overall pattern of births in Colorado which is drawn on two scales for clarity. The equation given for the top line shows that whilst the birth rate in Colorado fluctuates somewhat over the study period there is an overall decline of 159 births per years over the study period, albeit the detailed pattern is somewhat irregular. It is important to bear this in mind in considering the following graphs showing numbers of defects and rates.

S3 shows Down's syndrome data from Western Australia. This slide makes it very clear that whilst the rate of Downs syndrome born as live births is declining somewhat, the termination rate for this anomaly has risen markedly, so that their sum shows a clear upward trend. This important graph clearly underscores the critical role played by the applicable termination data in interpreting the trend lines under consideration. One notes that the termination data for Colorado for the present defects is believed not to be available at the time of writing.

On the basis of this graph it may be that the effects described below are as much as one half to one third of their total level net of the effect of therapeutic termination – although the level of this is obviously highly defect specific.

S4 introduces a title slide for this section.

S5 shows a very important slide which graphs the numbers and rates for all major congenital anomalies. It shows a clear upward trend for both numbers and rates. The raw data is given in the table to the right hand side. The numbers show a 69% rise across this fourteen year period, whilst the rates show a 70% rise. This annualizes to approximately 4.93% annual rate of rise for numbers and a 5.01% annual rate of rise for rates. Maintained over a 14 year period this is a not insignificant increase in the health burden to both individuals and the health system which treats these significant inborn defects.

There is also a rich literature linking antenatal cannabis use with cardiovascular defects [1-6], and a statement from the combined American Heart Association and American Academy of Pediatrics acknowledging that there is a causal link between cannabis and congenital heart disease [7].

S6 shows these rates as a percentage including the data on the graph.

The graphs in S7 show a significant rise in the rate of congenital heart disease. The equation on the upper graph shows an additional 40 cases per year (line slope). Both the numbers and rates of congenital heart disease are rising by about 4.5% annually, and about 61% over the whole period.

Ventricular Septal Defect (VSD) is also linked with cannabis use [1,6,7]. S8 shows that this is rising by about 6 cases annually, 35% overall, and about 2.5% annually.

S9 illustrates trends in the ostium secundum Artrial Septal Defect (ASD) which has previously been linked with cannabis exposure [6,7]. This is noted to be rising by about 46 cases annually; to have increased 260% over the whole period and to be rising at 18% annually. Indeed one also notes that the linear regression line accounts for 89% of the variance of the data. This implies that the rising trend is a strong and dominant factor in this trend line.

S10 shows data for microcephaly. One notes and average of 2 extra cases annually, a 96% rise over the 14 year period, and an annual rate of rise of 7%.

Chromosomal abnormalities have been reported as being associated with antenatal cannabis use. The data in S11 shows a increase of 3 cases per year, of 28% over the whole period and of 2% annually.

S12 introduces a summary slide for some of the selected stationary trends.

Many of the trends for congenital defects in Colorado are essentially stationary. Such data is shown for Cleft lip with or without cleft palette in S13, and for combined abdominal wall defects in S14. Several of the other defects which were inspected also appeared to be showing no real time dependent change or to occur at such low level that their trends are not stable. One notes in particular that gastroschsis, a defect which has been strongly linked with cannabis use in many studies [6,8-14] does not have data presented separately for it on the Colorado Health Information Dataset site at this time.

S15 presents a title slide for the cumulative and summative effect.

S16 shows a simple method, carry-forward projection for analyzing historcial trends. This is done first for births. The birth rate in the first 1-2 years (whichever is the lower) is simply carried forwards as if it had not changed in any of the subsequent years. The actual birth rate is listed in the second column. The difference appears in the fourth column and is the difference from the expected rate had the historical trends been simply continued along.

These various columns are then summed at their base as shown. One notes that an extra 33,311 births occurred than would have been expected, representing a 3.6% increase in births over this historical period, which annualizes to a 0.26% increase per year.

S17 shows the trend for all major congenital birth defects. This slide shows that whereas 67,620 would have been expected based on the historical trend, in fact 87,772 were observed, an excess of 20,152 cases or 29.8%.

S18 performs a similar calculation for all major cardiovascular defects and finds a 37% excess caseload.

S19 performs a similar function and finds a 17% excess for VSD.

S20 does the same function for ostium secundum ASD and finds a 98% excess caseload.

S21 shows a 30% excess for Microcephaly. The significance of this finding in a Zika virus era will I am sure not be lost on you.

S22 shows the data for the combined chromsomal anomalies and finds a 28% excess caseload.

S23 introduces a title slide for the final Summary section.

S24 shows the apparently very close correlation between all major congential anoamlies and cannabis use by various age groups in Colorado, as taken from the SAMHSA NSDUH survey at https://www.samhsa.gov/data/population-data-nsduh/reports?tab=38 .

S25 Shows the key graph again with its data included.

S26 presents the output of the R statistical analystical software showing the correlation coefficient, R=0.953852 and P = 0.00006594.

S27 presents another correlation calculation this time with the young adult rate of cannabis use again from the NSDUH SAMHSA survey (Data given in S24). In this study R=0.9254789 and P = 0.00003457.

S28 shows similar data with the major anomaly rate compared to the cannabis use rate in all Colorado dwellers over the age of 12 years. R=0.8825038 and P = 0.00002936.

S29 again shows this key graph.

S30 shows a final slide which summarizes all of the above information in a single table. The first column lists the various rising defects which have been considered. The second column shows the numbers of actual cases observed over the study period. The third column shows the number which would have been expected had the baseline trend been simply projected forwards. The fourth column gives the observed excess of cases for these defects. The fifth column shows the percentage rise over the entire period. The first line shows the numbers of births which forms the baseline trend against which the other categories are compared. The numbers of births rose 3.6% in the period 2000-2013. The other anomalies are compared with the rise in births to calculate the final column as a multiplicand of the baseline increase in birth numbers.

As noted above, this factor is believed to be a lower bound baseline since it is expected that for many of these defects foetal wastage would have occurred either by natural spontaneous miscarriage or by induced therapeutic termination of pregnancy, as indicated in Slide 3.

Conclusion

Hence these data indicate a signficant rise in the official numbers of major congenital anomalies in Colorado over the period when cannabis was gaining in popularity and into the very start of its medical legalization. Hence the figures are believed to be an underestimate of the cannabis related effect. They would almost certainly be substantially increased were data on therapeutic and other termination of pregnancy to become available. Hence these estimates included in the final table on S23 can only been seen as estimating the lower bound of the cannabis effect. Since the net effect shows an increase of 30% of all major defects, this can only be interpreted as a finding generating significant concern.

Matters of attributable risk effect arise in terms of interpreting how much of the increase might properly be attributed to cannabis itself and how much to various other extraneous and unknown confounding causes. Given that there is a published literature relating cannabis to all of these identified anomalies it seems likely that some significant fraction of the 20,152 excess cases can well be laid at the feet of cannabinoids. One notes also that these patients are exposed to mixed cannabinoids as occur in natural and cultured cannabis, including tetrahydrocannabinol, cannabidiol, cannabinol, cannabichromene, cannabiverin and many others so that all of them are potentially implicated on epidemiological grounds. Moreover many studies implicate multiple cannabinoids including cannabidiol in both genotoxic [15-24] and arteriopathic and / or arteritic [25-65] pathways.

The above cited literature links both maternal and paternal cannabis exposure [4] to teratological outcomes particularly congenital heart disease which is also the commonest of the major foetal malformations. The above citations also demonstrate significant multiple and complex interactions between cannabinoids and the cardiovascular system. Thus there are multiple potential mechanistic pathways from cannabis exposure to foetal pathology.

It was considered at the present time that it was important to bring these data to your attention as they are likely of significant public health import, particularly when amplified up to the national level. This is particularly so if, as is now a matter of record, cannabis use is becoming more common [64,66], if cannabis itself is becoming more concentrated as has also been amply documented [64] and if the major effect of therapeutic abortion is also included as seems only proper [67].

Please feel free to call on me if you would like further information concerning the research to which I have referred.

Yours sincerely,

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Australia.

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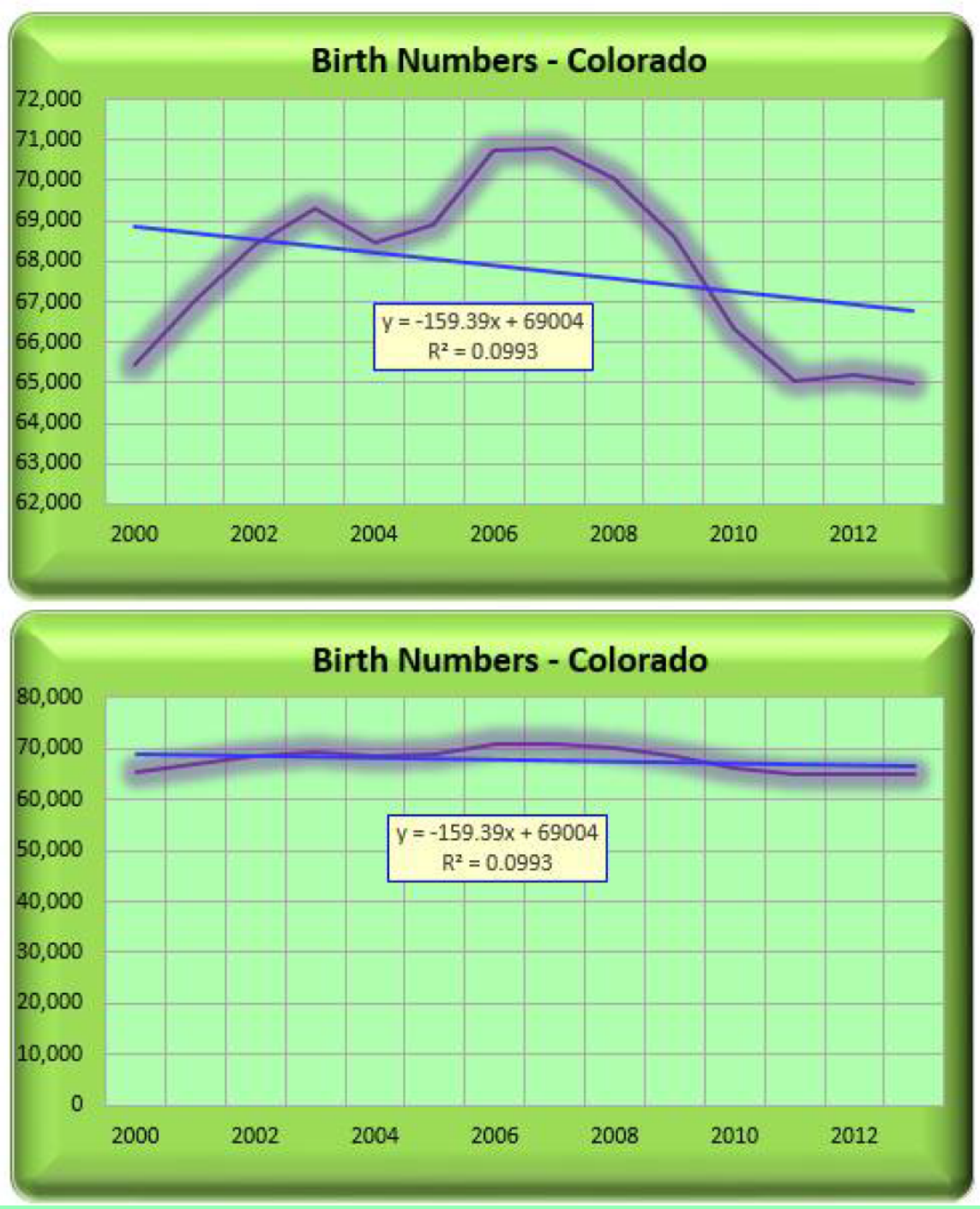
Congenital Anomalies Colorado

2000-2013

http://www.chd.dphe.state.co.us/cohid/
http://www.cohid.dphe.state.co.us/scripts/htmsql.exe/CrcsnPub.hsql

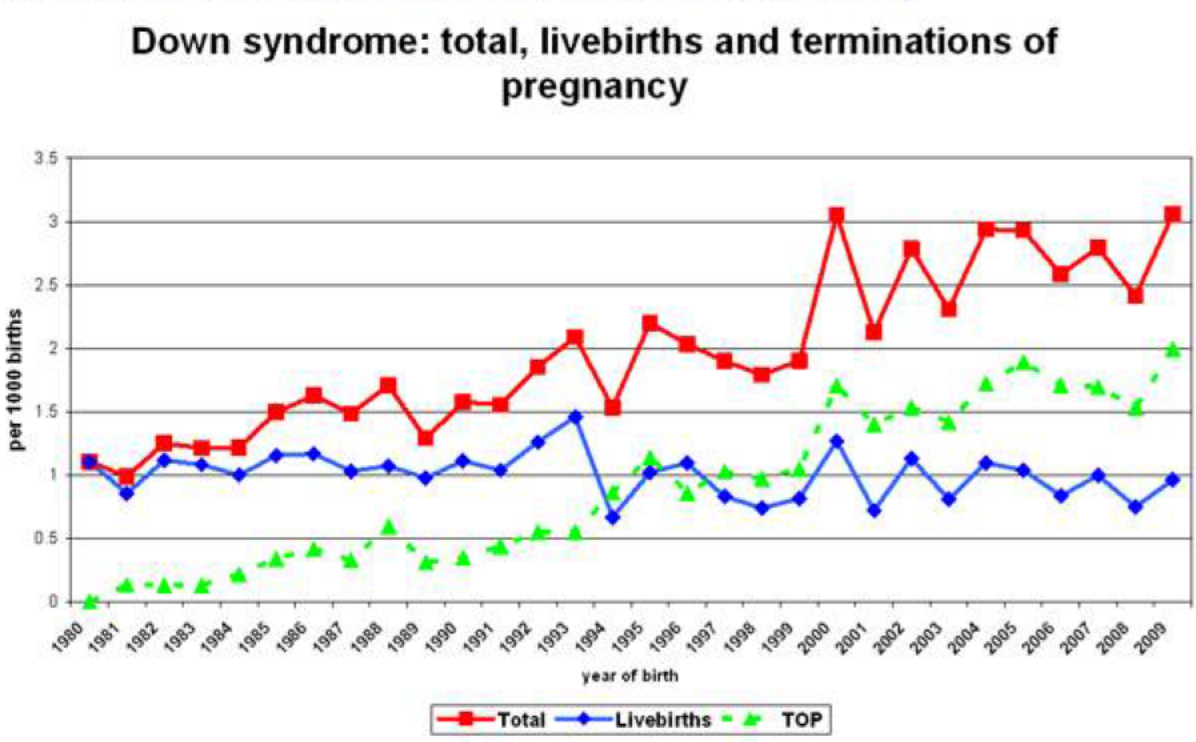
Births



http://www.chd.dphe.state.co.us/cohid/
http://www.cohid.dphe.state.co.us/scripts/htmsql.exe/CrcsnPub.hsql

Down's Syndrome in Western Australia

8. Down Syndrome: total, live births and terminations of pregnancy



http://www.kemh.health.wa.gov.au/services/register_developmental_anomalies/monitoring_trends.htm

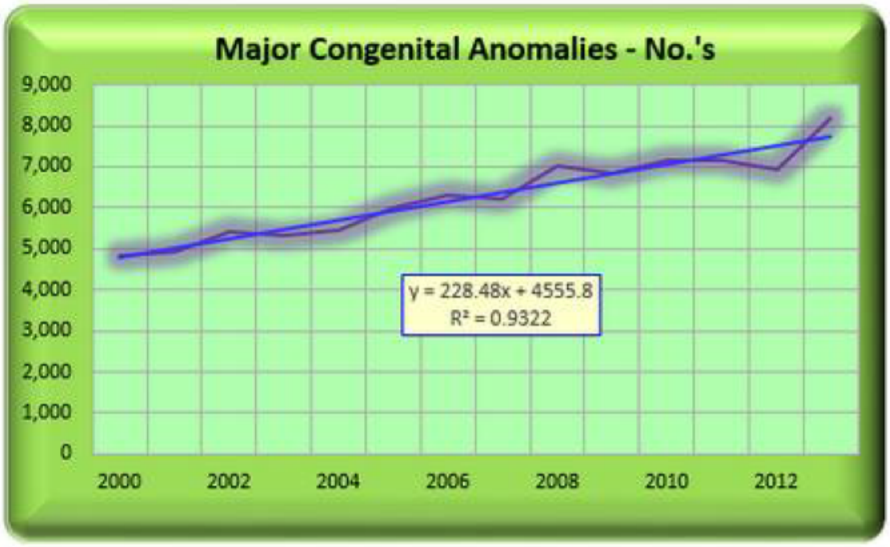
Rising Trends

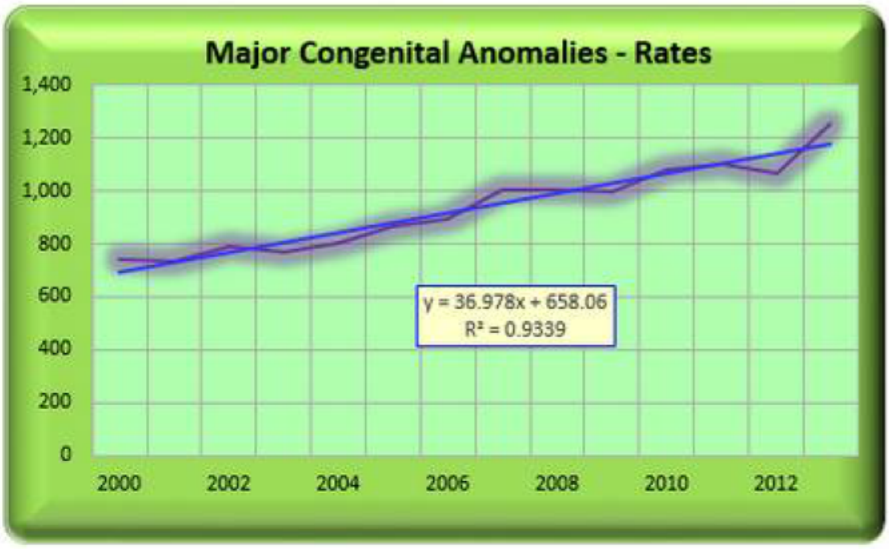
Colorado

2000-2013

http://www.chd.dphe.state.co.us/cohid/
http://www.cohid.dphe.state.co.us/scripts/htmsql.exe/CrcsnPub.hsql

Major Congenital Anomalies





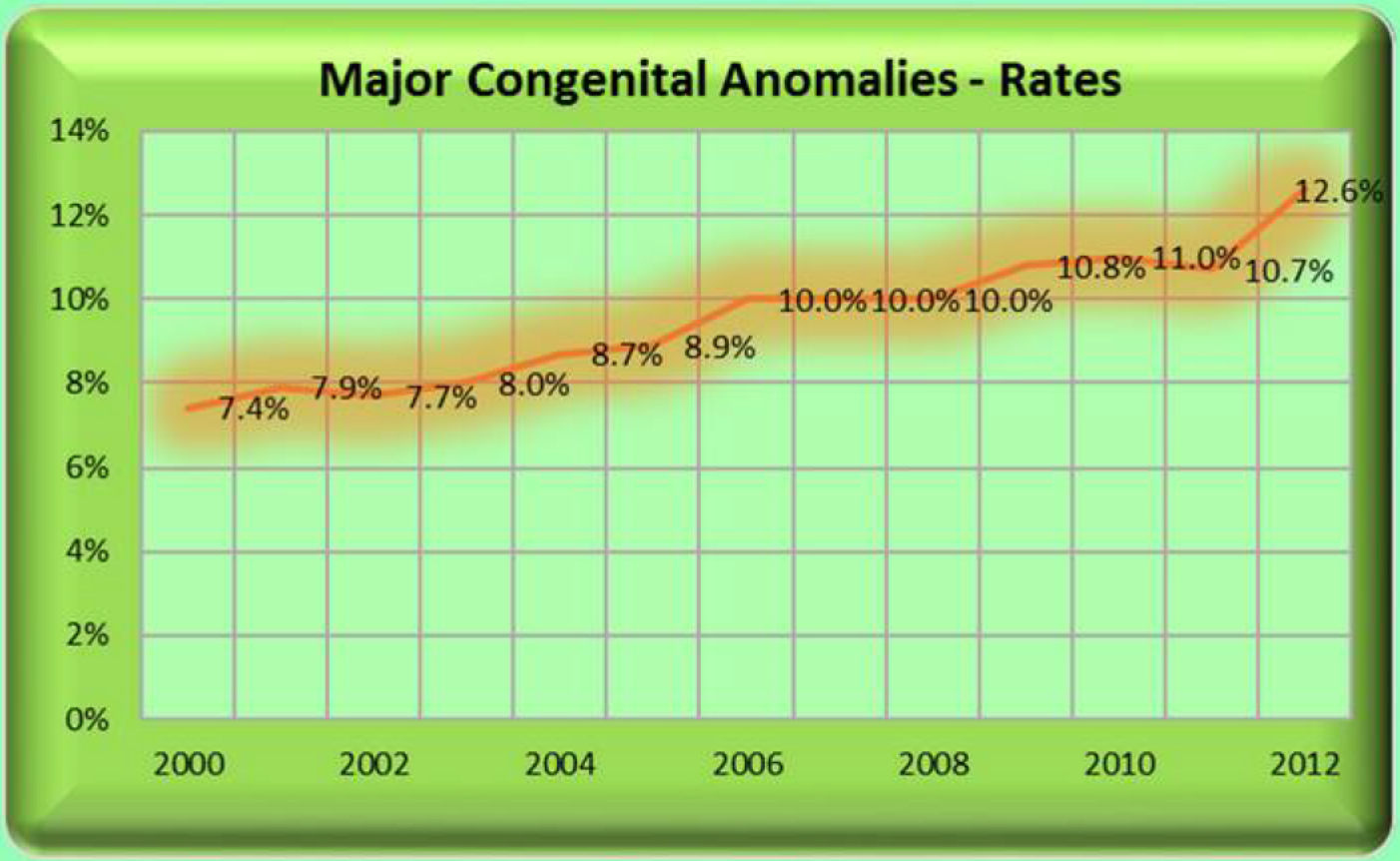
Major Congenital Anomalies

Rates/ 10,000 Live births
(Excluding Terminations)

Year	Majors	Majors Rate
2000	4830	738.2
2001	4942	737.5
2002	5406	790.1
2003	5311	766.3
2004	5482	800.6
2005	5978	867.4
2006	6325	894.2
2007	6213	1001.0
2008	7010	1001.0
2009	6826	995.0
2010	7171	1080.8
2011	7174	1102.8
2012	6939	1064.5
2013	8165	1256.1
Rise %	69.04%	70.16%
Annualized	4.93%	5.01%

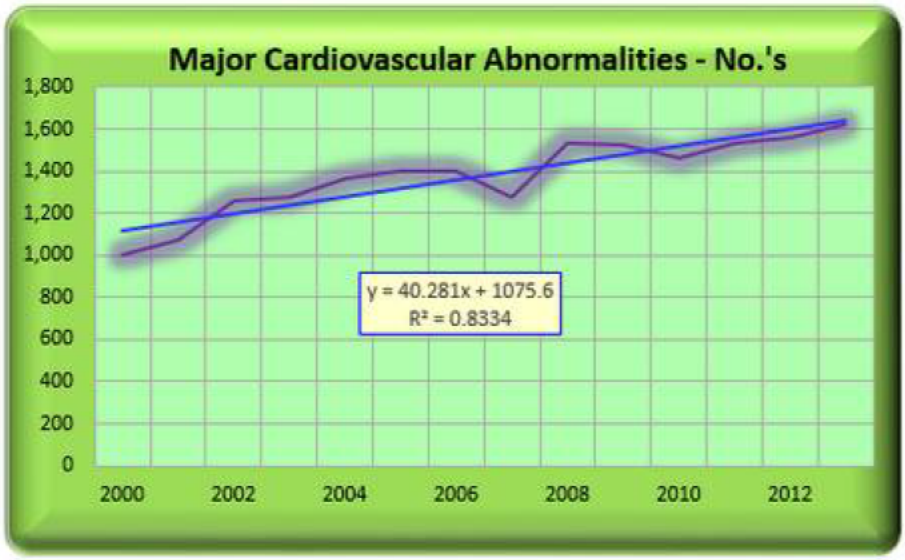
http://www.chd.dphe.state.co.us/cohid/
http://www.cohid.dphe.state.co.us/scripts/htmsql.exe/CrcsnPub.hsql

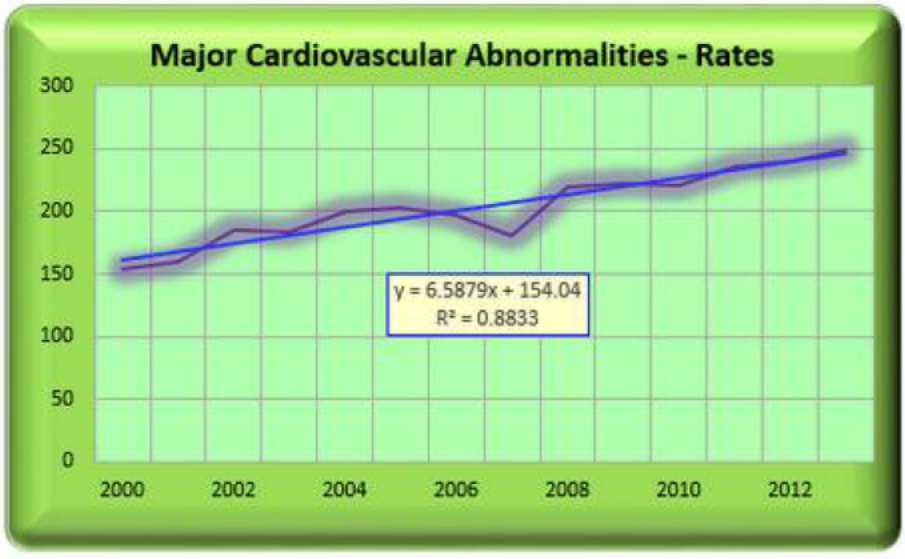
Major Congenital Anomalies as Percentage



http://www.chd.dphe.state.co.us/cohid/
http://www.cohid.dphe.state.co.us/scripts/htmsql.exe/CrcsnPub.hsql

Major Cardiovascular Anomalies





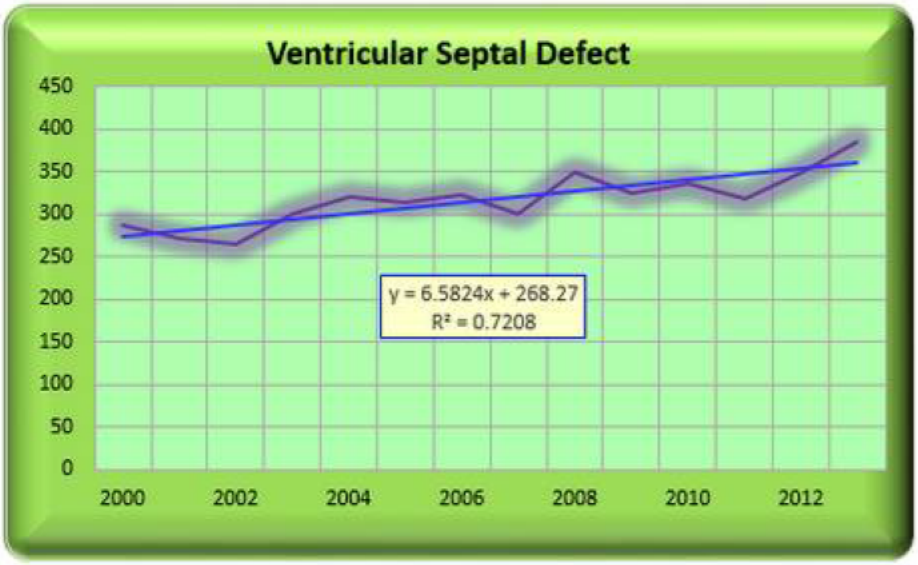
Major CVS Abnormalities

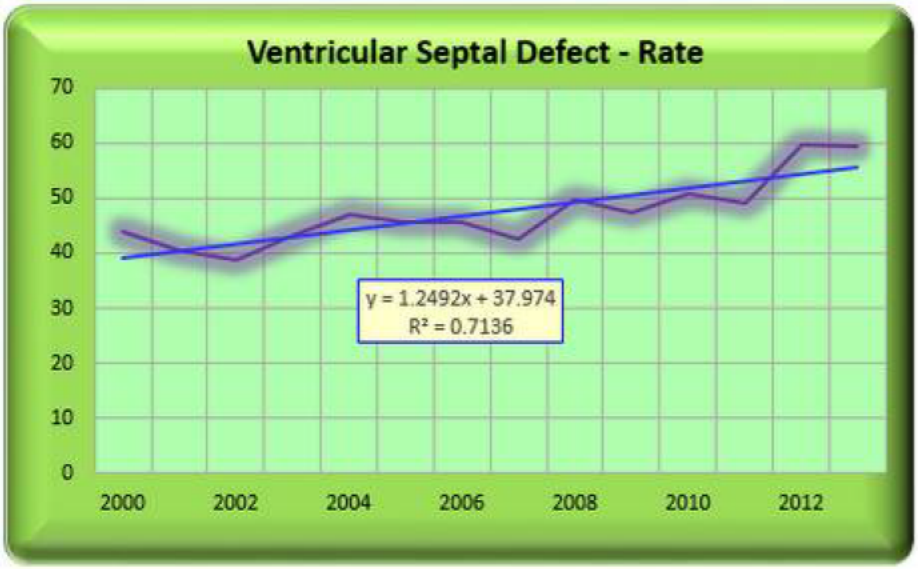
Rates/ 10,000 Live births
(Excluding Terminations)

Year	CVS	CVS Rate
2000	1002	153.1
2001	1071	159.8
2002	1263	184.6
2003	1273	183.7
2004	1368	199.8
2005	1398	202.8
2006	1397	197.5
2007	1274	179.9
2008	1530	218.5
2009	1528	222.7
2010	1464	220.7
2011	1536	236.1
2012	1562	239.6
2013	1622	249.5
Rise %	61.88%	62.97%
Annualized	4.42%	4.50%

http://www.chd.dphe.state.co.us/cohid/
http://www.cohid.dphe.state.co.us/scripts/htmsql.exe/CrcsnPub.hsql

Ventricular Septal Defect





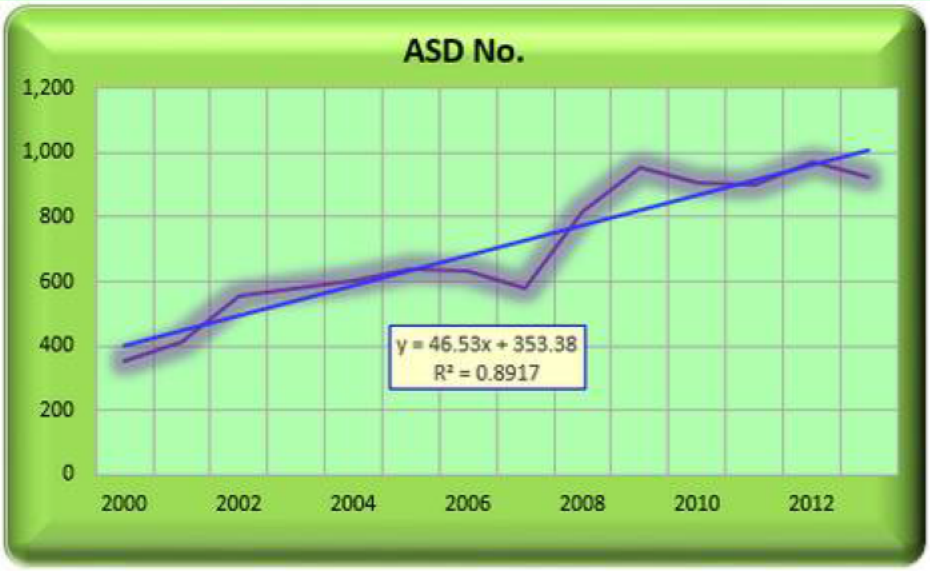
VSD

Rates/ 10,000 Live births
(Excluding Terminations)

Year	VSD	VSD Rate
2000	287	43.9
2001	271	40.4
2002	265	38.7
2003	300	43.3
2004	321	46.9
2005	315	45.7
2006	323	45.7
2007	300	42.4
2008	349	49.8
2009	324	47.2
2010	337	50.8
2011	319	49.0
2012	350	59.6
2013	386	59.4
Rise %	34.49%	35.31%
Annualized	2.46%	2.52%

http://www.chd.dphe.state.co.us/cohid/
http://www.cohid.dphe.state.co.us/scripts/htmsql.exe/CrcsnPub.hsql

Atrial Septal Defects - Ostium Secundum



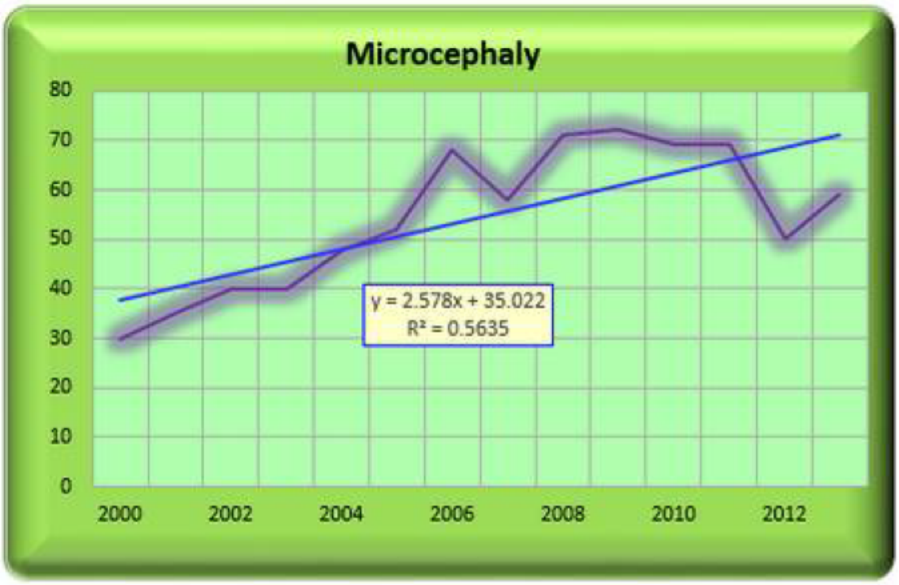
Ostium Secundum ASD's

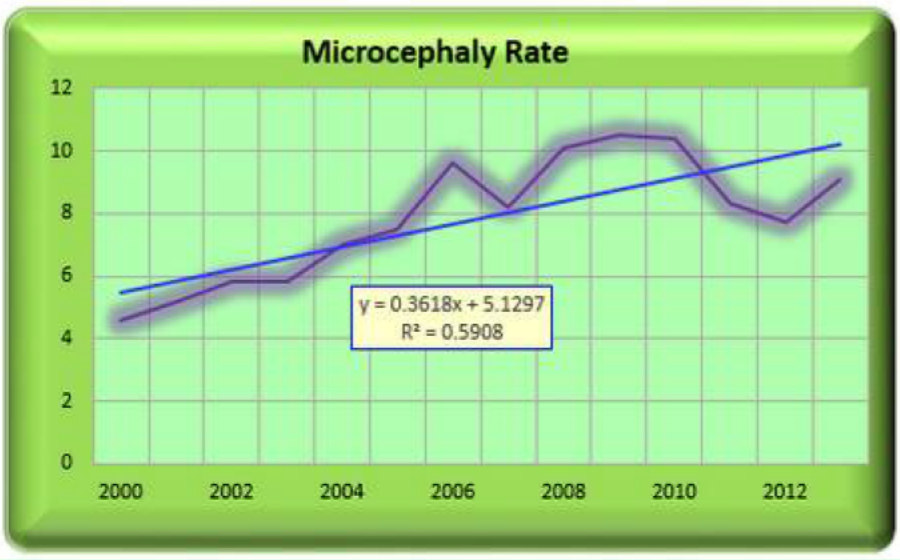
Rates/ 10,000 Live births
(Excluding Terminations)

Year	ASD No.	ASD - Rate
2000	355	54.3
2001	415	61.9
2002	554	81
2003	579	83.5
2004	606	88.5
2005	637	92.4
2006	635	89.8
2007	579	81.8
2008	815	116.4
2009	951	138.6
2010	909	137
2011	903	138.8
2012	969	148.6
2013	926	142.5
Rise %	260.85%	262.43%
Annualized	18.63%	18.75%

http://www.chd.dphe.state.co.us/cohid/
http://www.cohid.dphe.state.co.us/scripts/htmsql.exe/CrcsnPub.hsql

Microcephaly





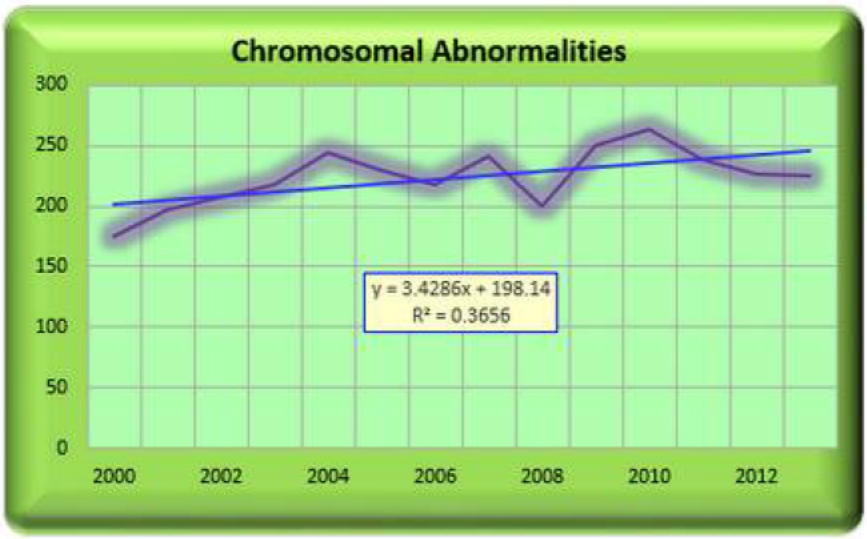
Microcephaly

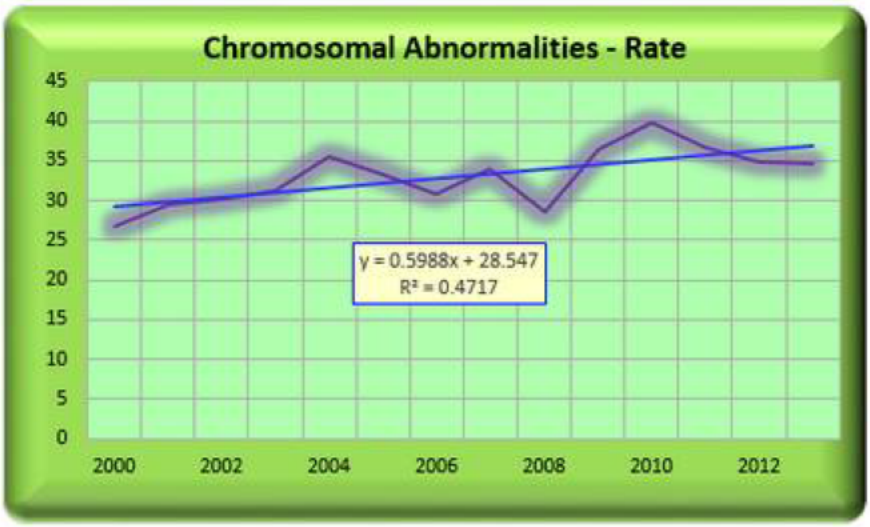
Rates/ 10,000 Live births
(Excluding Terminations)

Year	Microcephaly No.	Microcephaly Rate
2000	30	4.6
2001	35	5.2
2002	40	5.8
2003	40	5.8
2004	48	7
2005	52	7.5
2006	68	9.6
2007	58	8.2
2008	71	10.1
2009	72	10.5
2010	69	10.4
2011	69	8.3
2012	50	7.7
2013	59	9.1
Rise %	96.67%	97.83%
Annualized	6.90%	6.99%

http://www.chd.dphe.state.co.us/cohid/
http://www.cohid.dphe.state.co.us/scripts/htmsql.exe/CrcsnPub.hsql

Chromosomal Anomalies





Chromosomal Abnormalities

Rates/ 10,000 Live births
(Excluding Terminations)

Year	Chromosomal Abnormalities Number	Chromosomal Abnormalities Rate
2000	175	26.7
2001	197	29.4
2002	207	30.3
2003	217	31.3
2004	244	35.6
2005	230	33.4
2006	218	30.8
2007	241	34.0
2008	200	28.6
2009	250	36.4
2010	264	39.8
2011	239	36.7
2012	227	34.8
2013	225	34.6
Rise %	28.57%	29.41%
Annualized	2.04%	2.10%

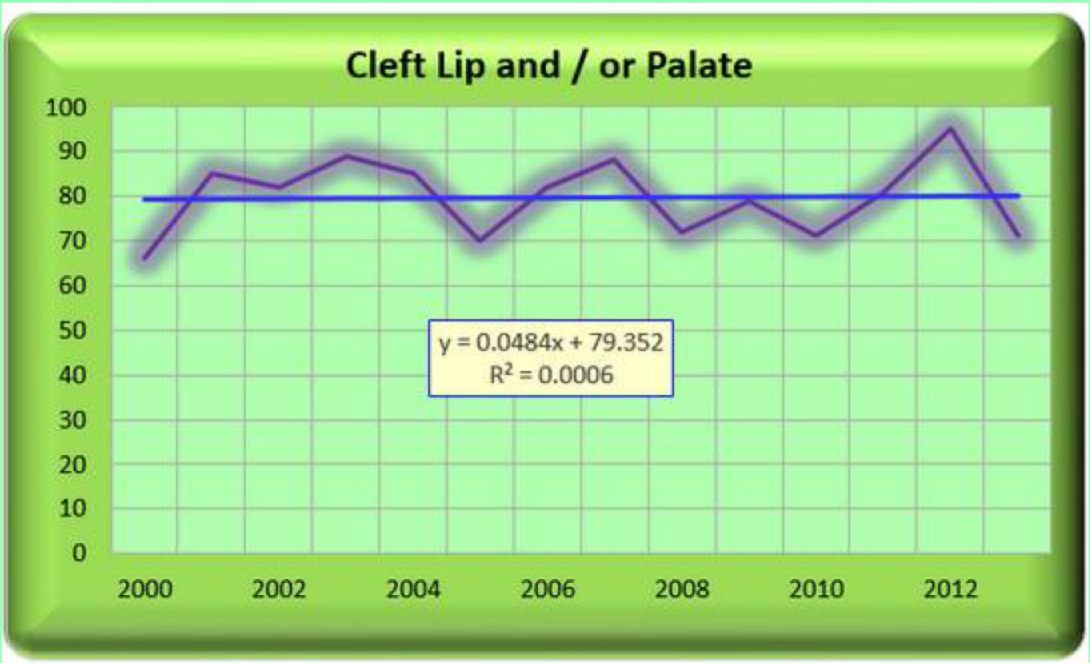
http://www.chd.dphe.state.co.us/cohid/
http://www.cohid.dphe.state.co.us/scripts/htmsql.exe/CrcsnPub.hsql

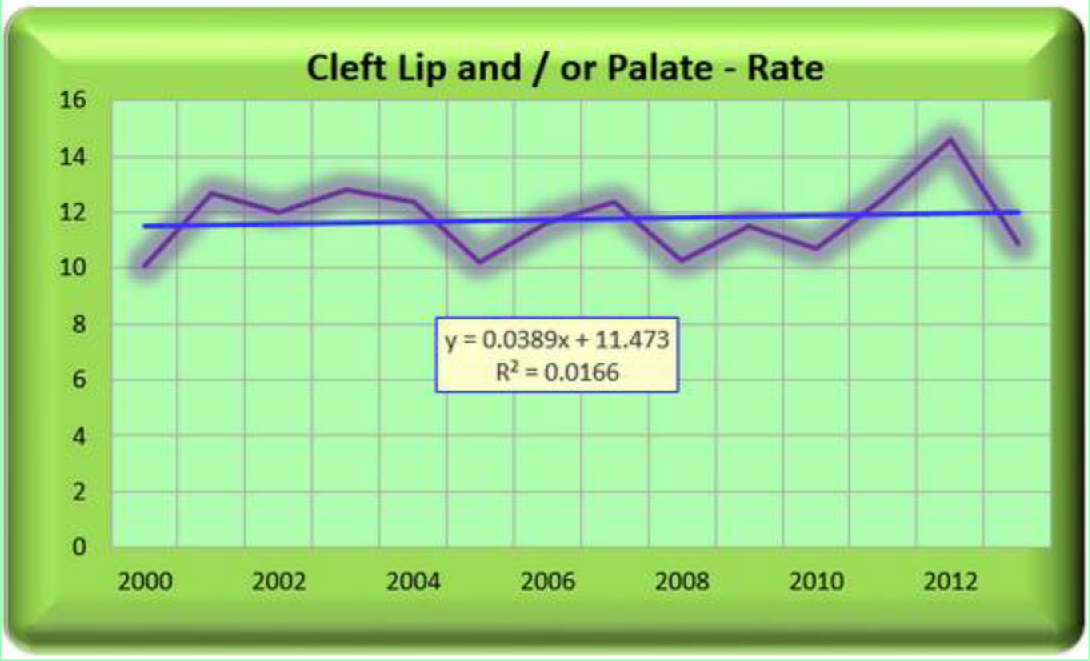
Stationary Time Trends

Colorado 2000-2013

http://www.chd.dphe.state.co.us/cohid/
http://www.cohid.dphe.state.co.us/scripts/htmsql.exe/CrcsnPub.hsql

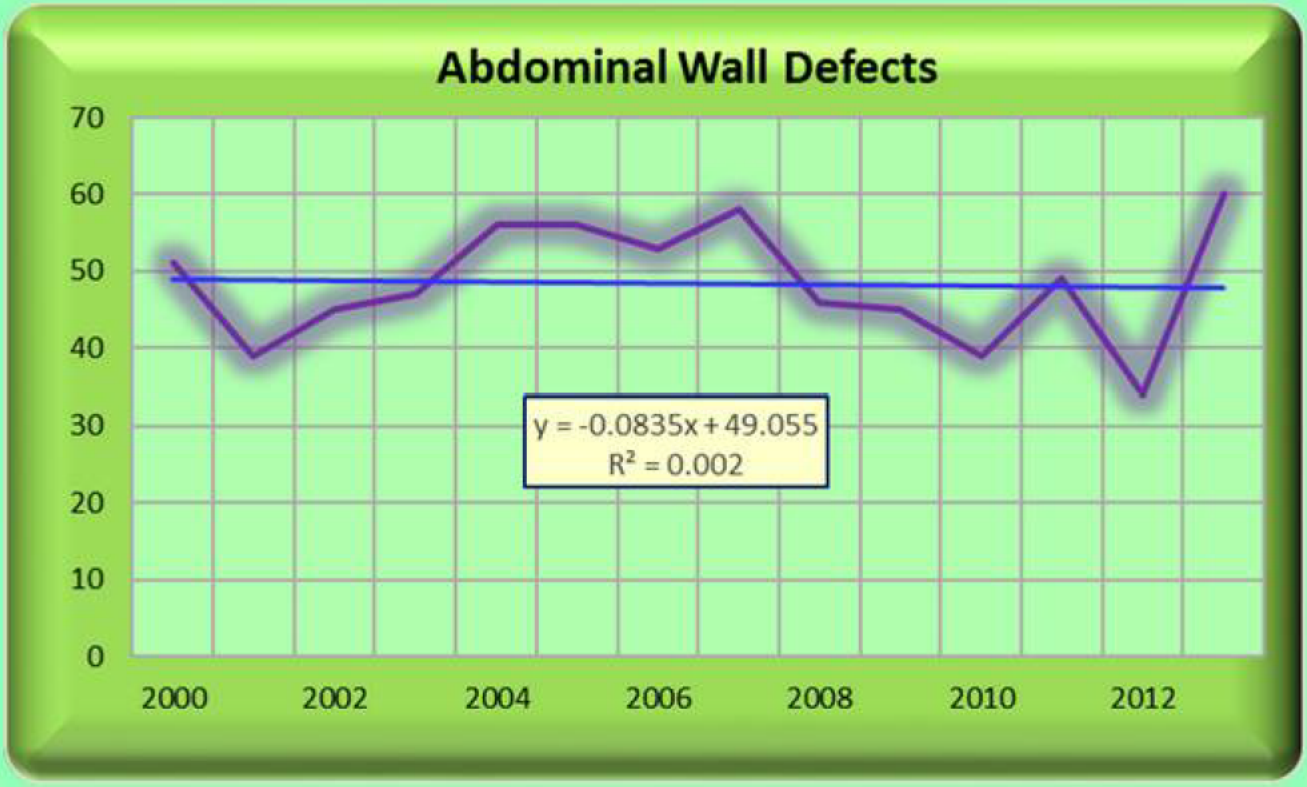
Cleft Lip +/- Palate





http://www.chd.dphe.state.co.us/cohid/
http://www.cohid.dphe.state.co.us/scripts/htmsql.exe/CrcsnPub.hsql

Abdominal Wall Defects



http://www.chd.dphe.state.co.us/cohid/
http://www.cohid.dphe.state.co.us/scripts/htmsql.exe/CrcsnPub.hsql

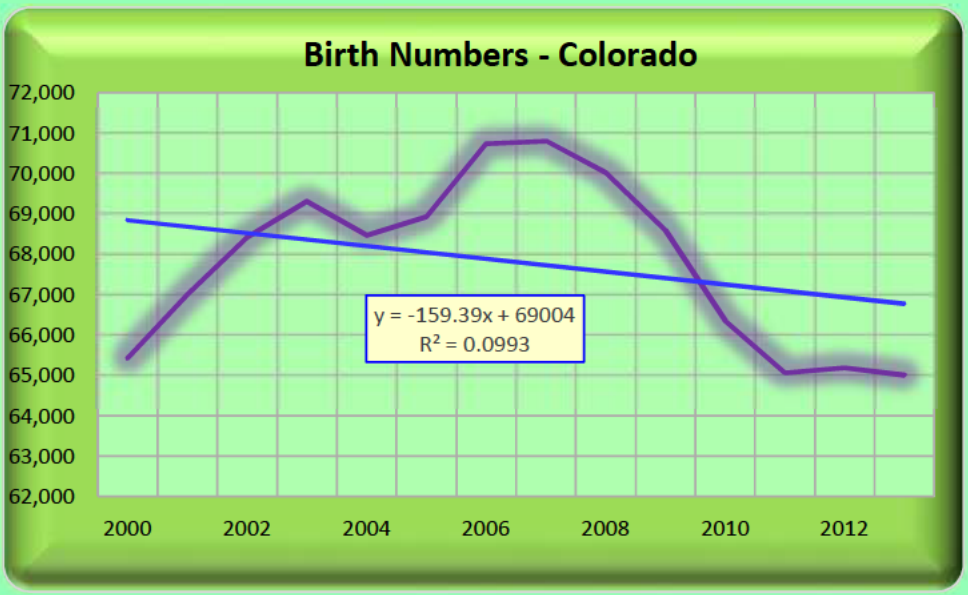
Cumulative Effects

Colorado

2000-2013

http://www.chd.dphe.state.co.us/cohid/
http://www.cohid.dphe.state.co.us/scripts/htmsql.exe/CrcsnPub.hsql

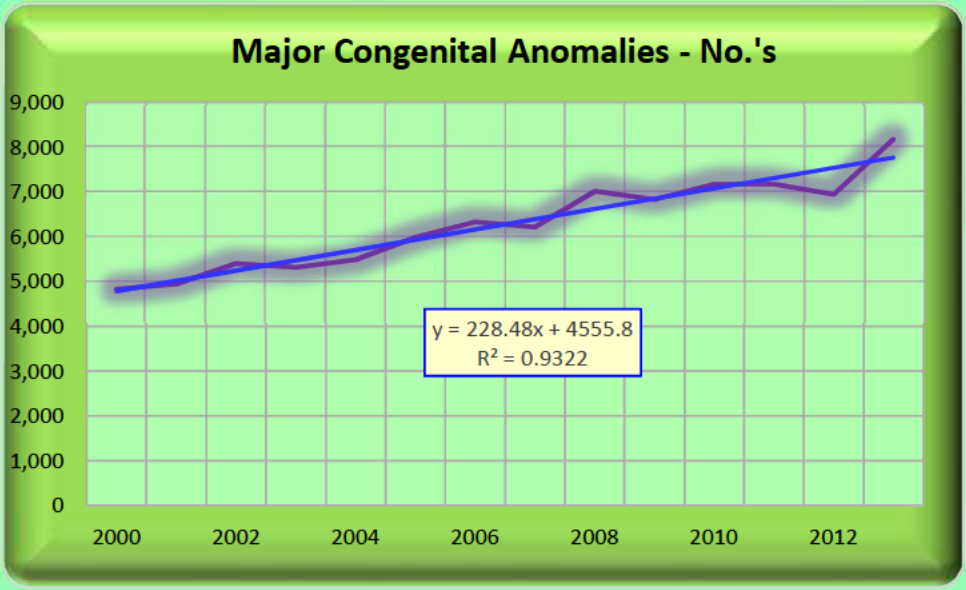
Cumulative Effects - Births



Year	Births	Projected	Difference
2000	65429	65429	0
2001	67006	65429	1577
2002	68420	65429	2991
2003	69304	65429	3875
2004	68475	65429	3046
2005	68922	65429	3493
2006	70737	65429	5308
2007	70804	65429	5375
2008	70028	65429	4599
2009	68602	65429	3173
2010	66346	65429	917
2011	65052	65429	-377
2012	65188	65429	-241
2013	65004	65429	-425
Cumulative	**949317**	**916006**	**33311**
% Change			**3.6%**
Annualized			**0.26%**

http://www.chd.dphe.state.co.us/cohid/
http://www.cohid.dphe.state.co.us/scripts/htmsql.exe/CrcsnPub.hsql

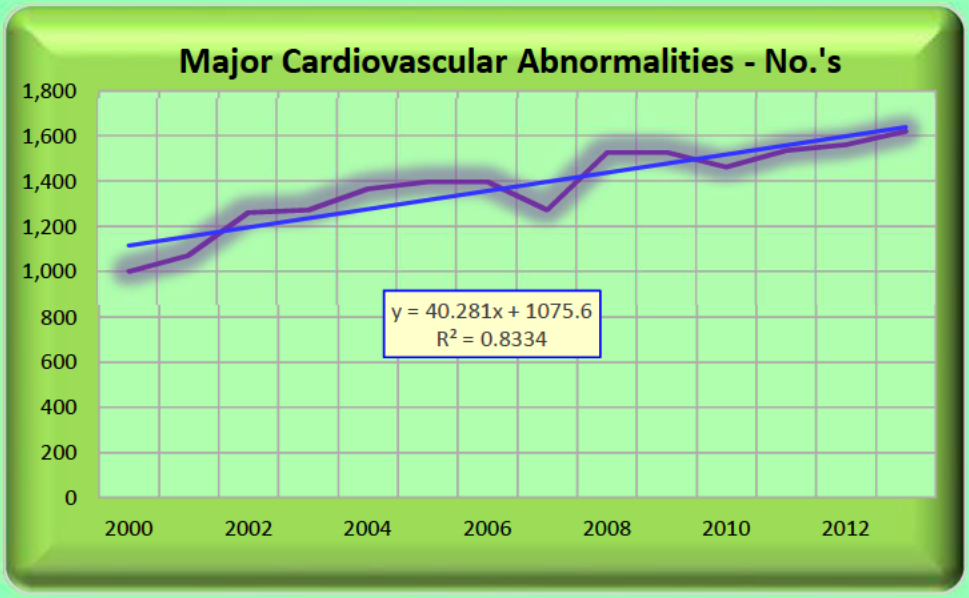
Cumulative Effects - All Major Defects



Year	Majors	Projection	Difference
2000	4830	4830	0
2001	4942	4830	112
2002	5406	4830	576
2003	5311	4830	481
2004	5482	4830	652
2005	5978	4830	1148
2006	6325	4830	1495
2007	6213	4830	1383
2008	7010	4830	2180
2009	6826	4830	1996
2010	7171	4830	2341
2011	7174	4830	2344
2012	6939	4830	2109
2013	8165	4830	3335
Cumulative	**87772**	**67620**	**20152**
% Change			**29.8%**

http://www.chd.dphe.state.co.us/cohid/
http://www.cohid.dphe.state.co.us/scripts/htmsql.exe/CrcsnPub.hsql

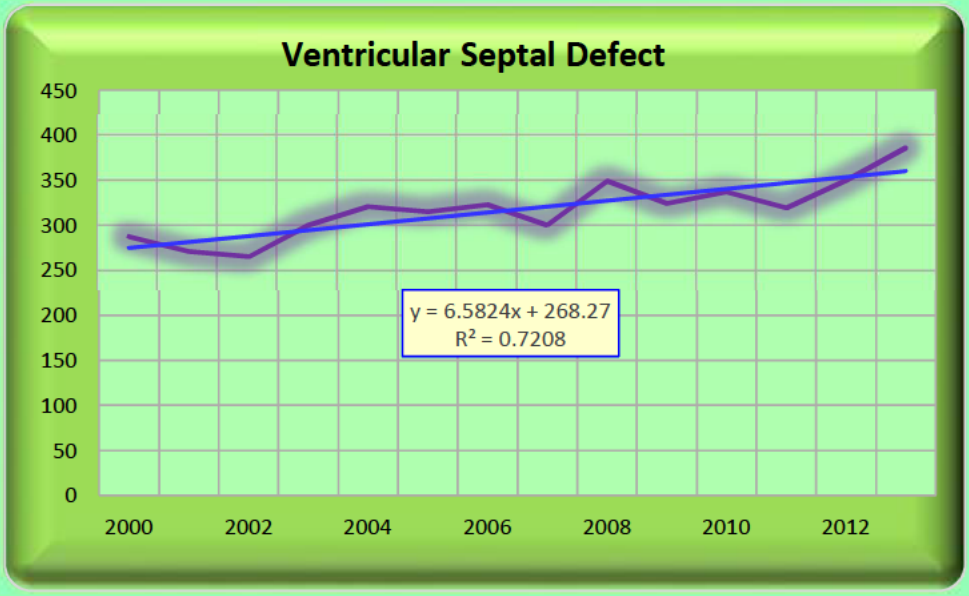
Cumulative Effects - All CVS Anomalies



Year	CVS	Projected	Difference
2000	1002	1002	0
2001	1071	1002	69
2002	1263	1002	261
2003	1273	1002	271
2004	1368	1002	366
2005	1398	1002	396
2006	1397	1002	395
2007	1274	1002	272
2008	1530	1002	528
2009	1528	1002	526
2010	1464	1002	462
2011	1536	1002	534
2012	1562	1002	560
2013	1622	1002	620
Cumulative	**19288**	**14028**	**5260**
% Change			**37.5%**

http://www.chd.dphe.state.co.us/cohid/
http://www.cohid.dphe.state.co.us/scripts/htmsql.exe/CrcsnPub.hsql

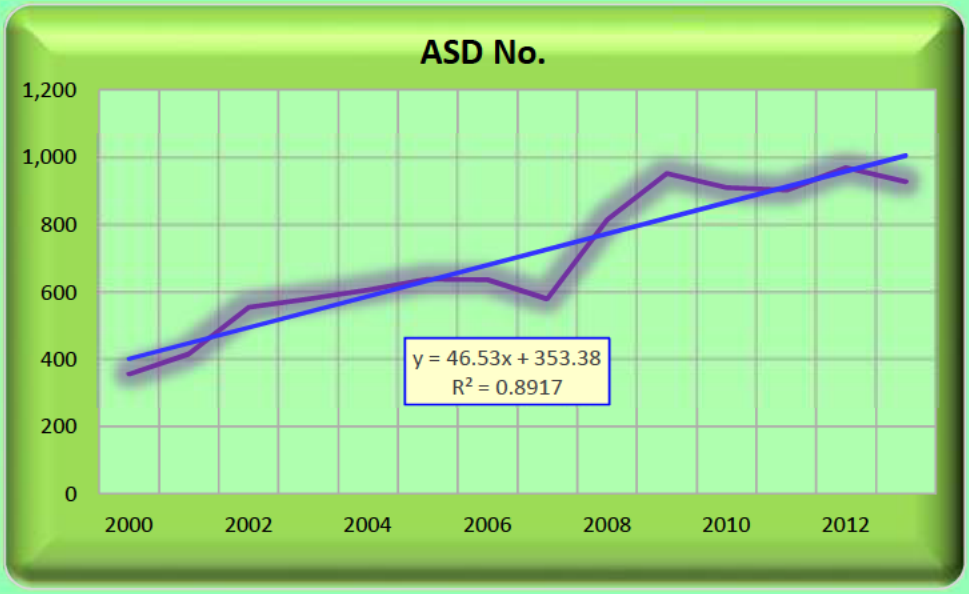
Cumulative Effects - VSD



Year	VSD	Projected	Difference
2000	287	271	16
2001	271	271	0
2002	265	271	-6
2003	300	271	29
2004	321	271	50
2005	315	271	44
2006	323	271	52
2007	300	271	29
2008	349	271	78
2009	324	271	53
2010	337	271	66
2011	319	271	48
2012	350	271	79
2013	386	271	115
Cumulative	**4447**	**3794**	**653**
% Change			**17.2%**

http://www.chd.dphe.state.co.us/cohid/
http://www.cohid.dphe.state.co.us/scripts/htmsql.exe/CrcsnPub.hsql

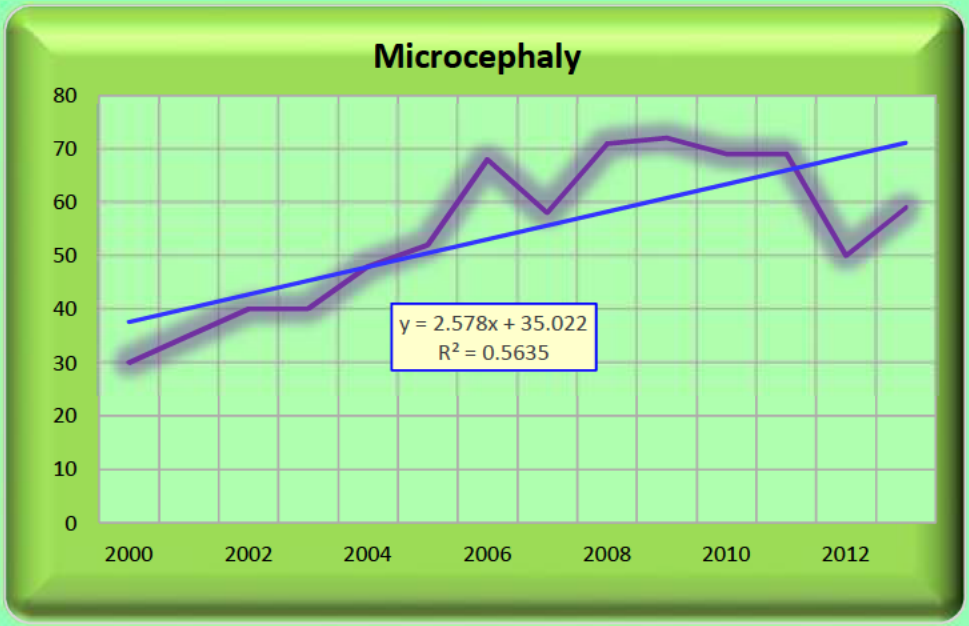
Cumulative Effects - ASD - Secundum



Year	ASD No.	Projection	Difference
2000	355	355	0
2001	415	355	60
2002	554	355	199
2003	579	355	224
2004	606	355	251
2005	637	355	282
2006	635	355	280
2007	579	355	224
2008	815	355	460
2009	951	355	596
2010	909	355	554
2011	903	355	548
2012	969	355	614
2013	926	355	571
Cumulative	**9833**	**4970**	**4863**
% Change			**97.8%**

http://www.chd.dphe.state.co.us/cohid/
http://www.cohid.dphe.state.co.us/scripts/htmsql.exe/CrcsnPub.hsql

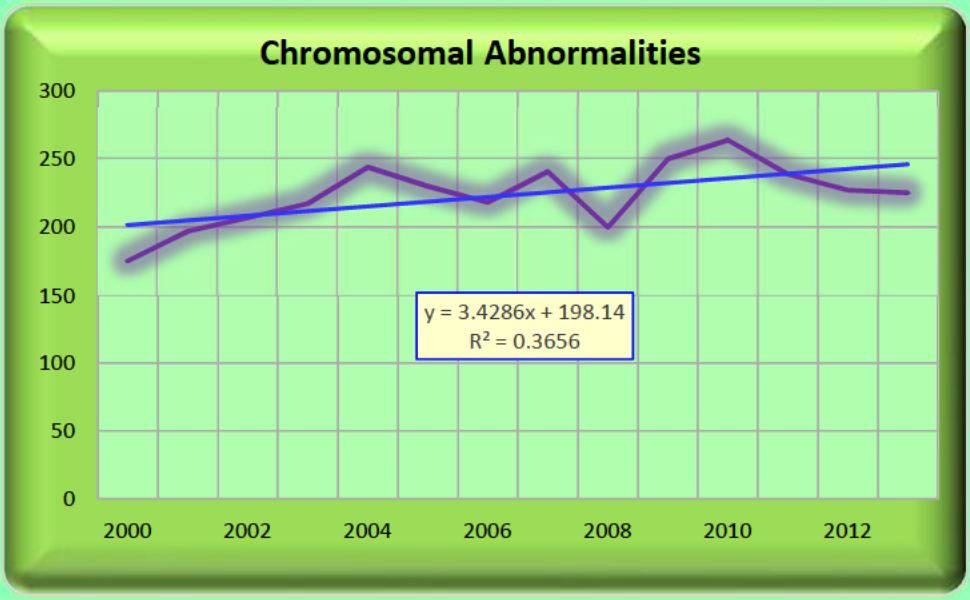
Cumulative Effects - Microcephaly



Year	Majors	Projection	Difference
2000	4830	4830	0
2001	4942	4830	112
2002	5406	4830	576
2003	5311	4830	481
2004	5482	4830	652
2005	5978	4830	1148
2006	6325	4830	1495
2007	6213	4830	1383
2008	7010	4830	2180
2009	6826	4830	1996
2010	7171	4830	2341
2011	7174	4830	2344
2012	6939	4830	2109
2013	8165	4830	3335
Cumulative	**87772**	**67620**	**20152**
% Change			**29.8%**

http://www.chd.dphe.state.co.us/cohid/
http://www.cohid.dphe.state.co.us/scripts/htmsql.exe/CrcsnPub.hsql

Cumulative Effects - Chromosomal Abnormalities



Year	Chromosomal Abnormalities Number	Projection	Difference
2000	175	175	0
2001	197	175	22
2002	207	175	32
2003	217	175	42
2004	244	175	69
2005	230	175	55
2006	218	175	43
2007	241	175	66
2008	200	175	25
2009	250	175	75
2010	264	175	89
2011	239	175	64
2012	227	175	52
2013	225	175	50
Cumulative	**3134**	**2450**	**684**
% Change			**27.9%**

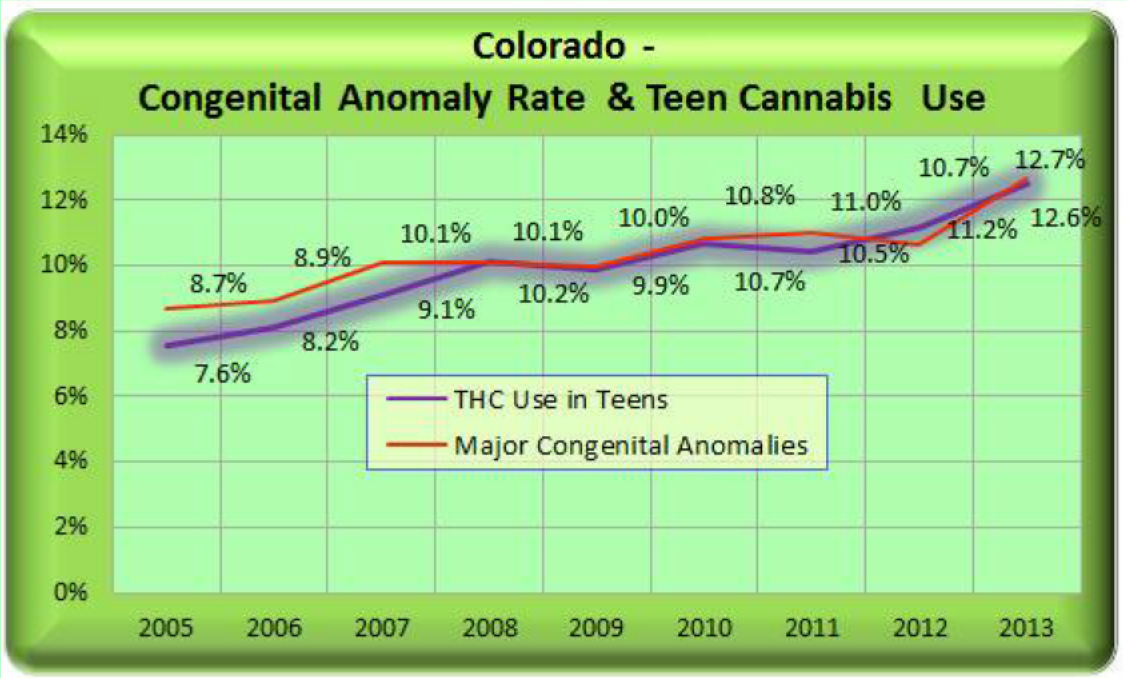
http://www.chd.dphe.state.co.us/cohid/
http://www.cohid.dphe.state.co.us/scripts/htmsql.exe/CrcsnPub.hsql

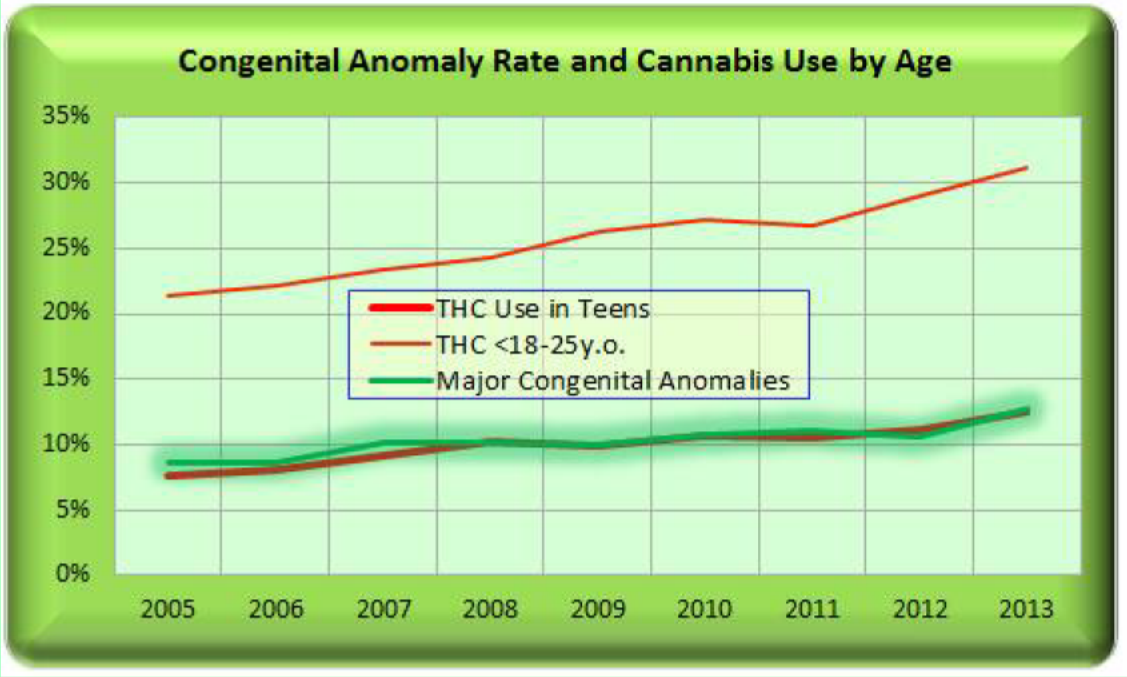
Overall Cumulative Summary

Colorado 2000-2013

http://www.chd.dphe.state.co.us/cohid/
http://www.cohid.dphe.state.co.us/scripts/htmsql.exe/CrcsnPub.hsql

Close Correlation between Cannabis Consumption and Congenital Anomalies Rates



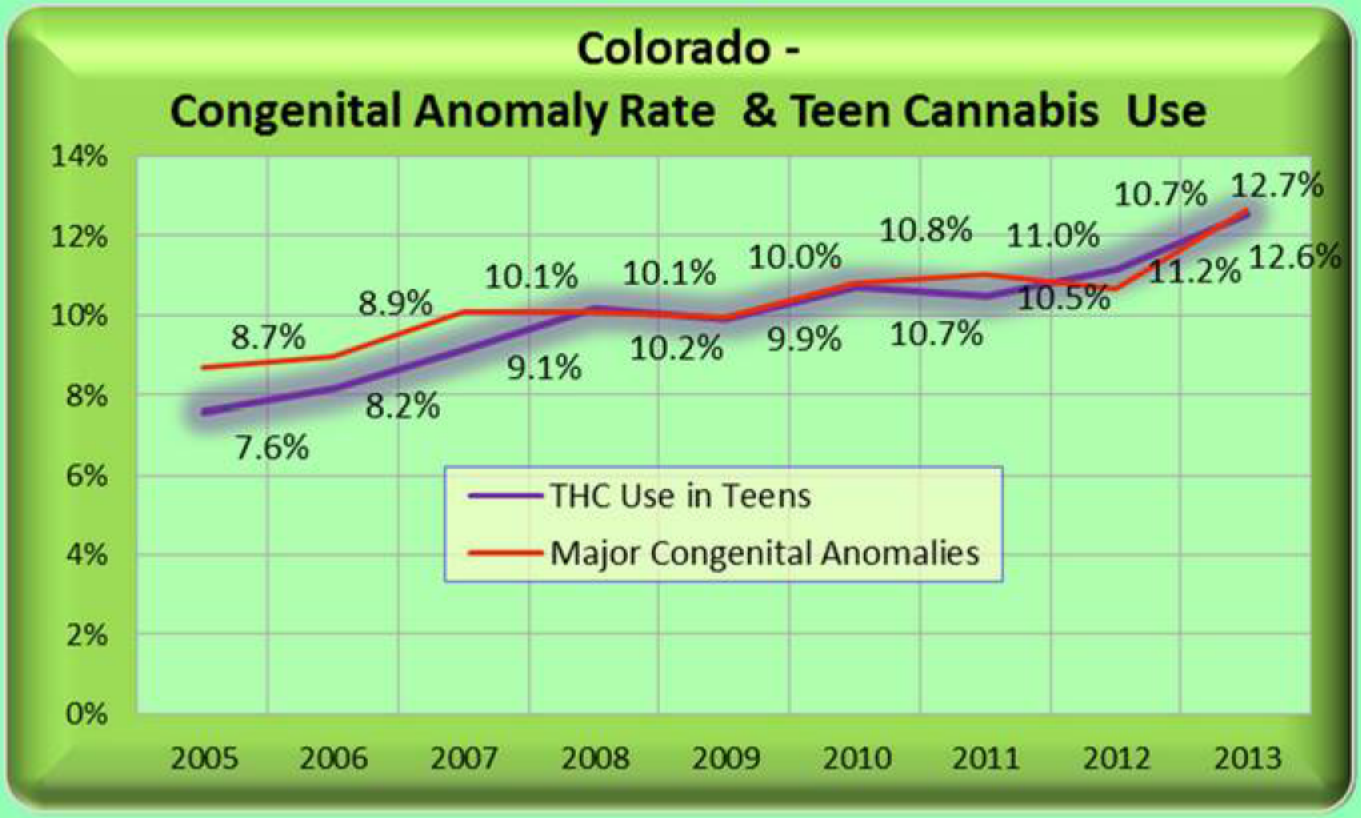


http://www.chd.dphe.state.co.us/cohid/

http://www.cohid.dphe.state.co.us/scripts/htmsql.exe/CrcsnPub.hsql

https://www.samhsa.gov/data/sites/default/files/NSDUH-FFR1-2016/NSDUH-FFR1-2016.pdf

Close Correlation between Cannabis Consumption and Congenital Anomalies Rates



http://www.chd.dphe.state.co.us/cohid/

http://www.cohid.dphe.state.co.us/scripts/htmsql.exe/CrcsnPub.hsql

https://www.samhsa.gov/data/sites/default/files/NSDUH-FFR1-2016/NSDUH-FFR1-2016.pdf

Correlation == 0.9539
P == 0.00006594

```
> cor.test (a,x, alternative="two.sided",
+           method="pearson", exact=TRUE, conf.level = 0.95)

        Pearson's product-moment correlation

data:  a and x
t = 8.4142, df = 7, p-value = 6.594e-05
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 0.7908924 0.9905319
sample estimates:
     cor
0.953952
```

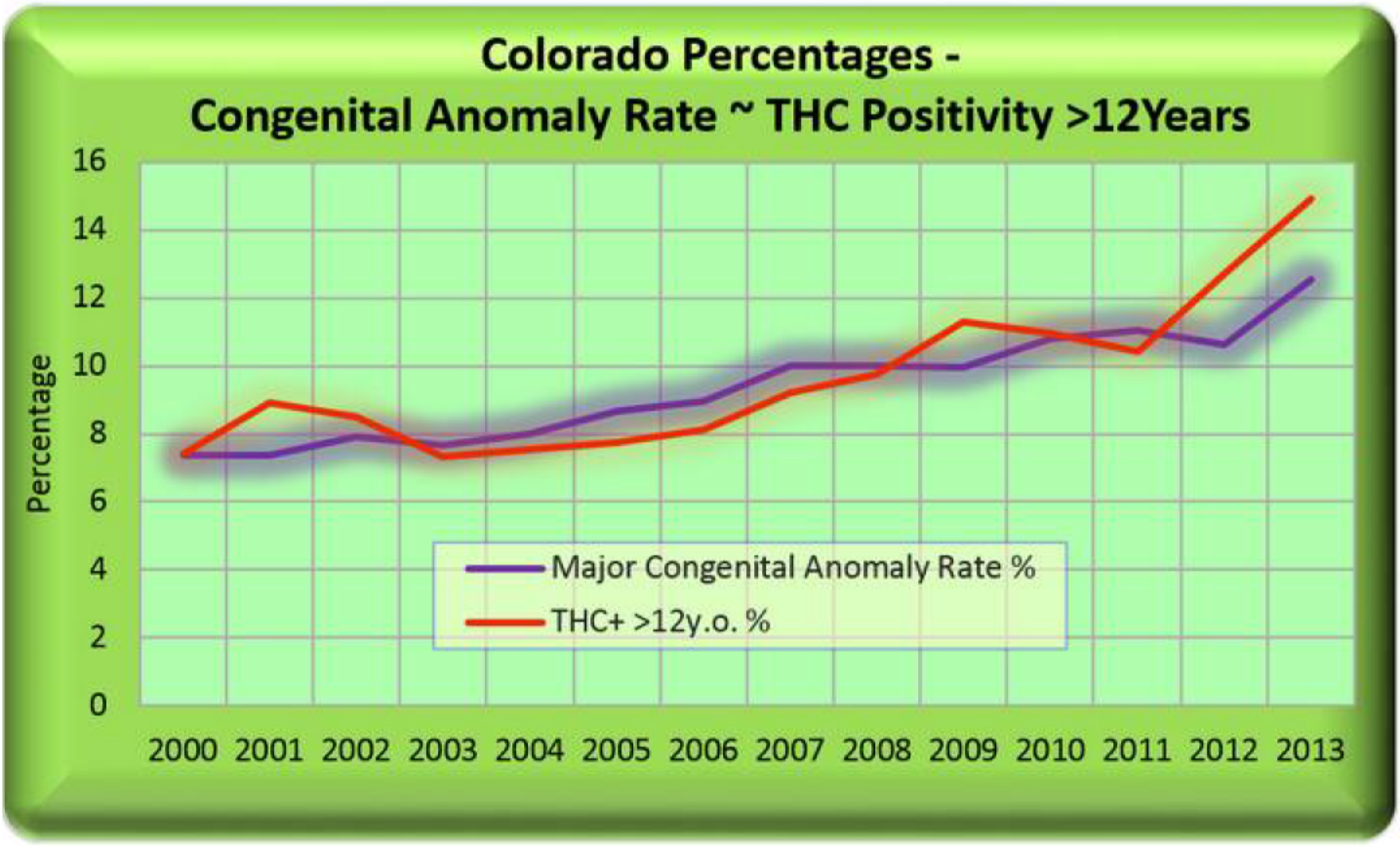
http://www.chd.dphe.state.co.us/cohid/
http://www.cohid.dphe.state.co.us/scripts/htmsql.exe/CrcsnPub.hsql
https://www.samhsa.gov/data/sites/default/files/NSDUH-FFR1-2016/NSDUH-FFR1-2016.pdf

Young Adult Correlation == 0.9258
P == 0.0003457

```
> CTD
  Year THCTeens THC18.25 MeanUse Majors
1 2005   0.0760   0.2143  0.1452 0.0867
2 2006   0.0815   0.2221  0.1518 0.0894
3 2007   0.0913   0.2344  0.1629 0.1001
4 2008   0.1017   0.2428  0.1723 0.1001
5 2009   0.0991   0.2635  0.1813 0.0995
6 2010   0.1072   0.2726  0.1899 0.1081
7 2011   0.1047   0.2681  0.1864 0.1103
8 2012   0.1116   0.2905  0.2011 0.1065
9 2013   0.1256   0.3124  0.2190 0.1265
>
> x <- CTD$THCTeens
> y <- CTD$THC18.25
> z <- CTD$MEanUse
> a <- CTD$Majors
>
> cor.test (a,y, alternative="two.sided",
+           method="pearson", exact=TRUE, conf.level = 0.95)

        Pearson's product-moment correlation

data:  a and y
t = 6.4639, df = 7, p-value = 0.0003457
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 0.6781881 0.9844974
sample estimates:
      cor
0.9254759
```

http://www.chd.dphe.state.co.us/cohid/
http://www.cohid.dphe.state.co.us/scripts/htmsql.exe/CrcsnPub.hsql
https://www.samhsa.gov/data/sites/default/files/NSDUH-FFR1-2016/NSDUH-FFR1-2016.pdf

Close Correlation between Cannabis Consumption and Congenital Anomalies Rates



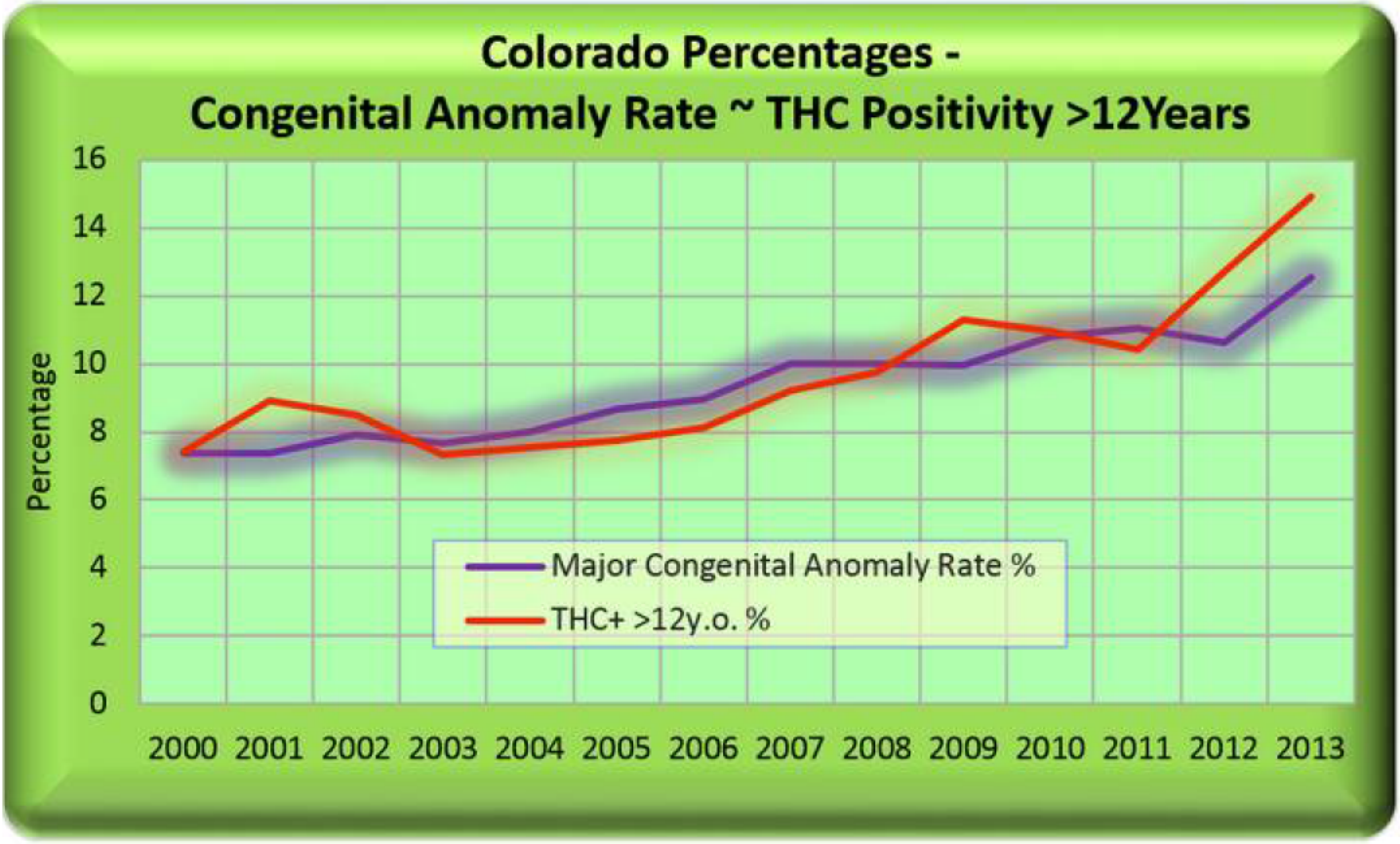

```
> cor.test (x,y, alternative="two.sided",
+           method="pearson", exact=TRUE, conf.level = 0.95)

        Pearson's product-moment correlation

data:  x and y
t = 6.5002, df = 12, p-value = 2.936e-05
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 0.6618084 0.9624345
sample estimates:
      cor
0.8825038
```

http://www.chd.dphe.state.co.us/cohid/

http://www.cohid.dphe.state.co.us/scripts/htmsql.exe/CrcsnPub.hsql

https://www.samhsa.gov/data/sites/default/files/NSDUH-FFR1-2016/NSDUH-FFR1-2016.pdf

Colorado Percentages - Congenital Anomalies Rates & Cannabis Consumption Rates >12 Years



www.samhsa.gov
http://www.chd.dphe.state.co.us/cohid/
http://www.cohid.dphe.state.co.us/scripts/htmsql.exe/CrcsnPub.hsql
https://www.samhsa.gov/data/sites/default/files/NSDUH-FFR1-2016/NSDUH-FFR1-2016.pdf

Cumulative Overall Effects

Anomaly	Cumulative Total 2000-2013	Projected Total from Baseline	Excess Above Baseline	% Change 2000-2013	Increase Relative to Births
Births	949,317	916,006	33,311	3.6%	1.00
Major Congenital Defects	87,772	67,620	20,152	29.8%	8.20
Major CVS	19,288	14,028	5,260	37.5%	10.31
VSD	4,447	3,794	653	17.2%	4.73
ASD-Secundum	9,833	4,970	4,863	97.8%	26.91
Microcephaly	761	420	341	81.2%	22.33
Chromosomal	3,134	2,450	684	27.9%	7.68

http://www.chd.dphe.state.co.us/cohid/
http://www.cohid.dphe.state.co.us/scripts/htmsql.exe/CrcsnPub.hsql