Environmetrical Approach to Analyze Endocrine Disruptors in the Environment and Human Body

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1. Introduction to POPs as Endocrine Disruptors

The most intractable pollutants are nuclear wastes, hazardous wastes (chemical substances), and wastes that threaten global biogeochemical processes, such as greenhouse gases, chemically the hardest to sequester or detoxify, physiologically the hardest for our senses to detect and economically and politically the most difficult to regulate [Meadows et al., 2004]. We focus in this paper on human-synthesized chemicals, called Persistent Organic Pollutants (POPs). Environmental and human exposure to Persistent Organic Pollutants (POPs) have been the subject of scientific investigation and political regulation for almost 40 years. The signature of the Stockholm Convention on Persistent Organic Pollutants in May 2001, and its amendment in 2010 [UNEP, 2011] is one of the most obvious and important results of the scientific and political discussion on global pollutants. This Convention on POPs is a global treaty to protect human health and the environment from chemicals that remain intact in the environment for long periods, become widely distributed geographically, accumulate in the fatty tissue of humans and wildlife, and have adverse effects to human health and to the environment. Exposure to POPs can lead to serious health effects including certain cancers, birth defects (like cryptorchidism), dysfunctional immune and reproductive systems, greater susceptibility to disease and even diminished intelligence. Given their long range transport, no one government acting alone can protect its citizens or its environment from POPs. The evaluation of the data is a major step in the elucidation of the danger of these chemicals. Currently 22 POPs are listed in the convention mentioned above. 20 of these chemicals are in the focus of our data evaluation approach. Most of these 20 organochlorine chemicals are regarded as endocrine disruptors.

An endocrine disrupter is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations [EC, 2011]. Some chemicals can act on the endocrine system to disturb the homeostatic mechanisms of the body or to initiate processes at abnormal times in the life cycle. The chemicals can exert their effects through a number of different mechanisms:

- They may mimic the biological activity of a hormone by binding to a cellular receptor, leading to an unwarranted response by initiating the cell's normal response to the naturally occurring hormone at the wrong time or to an excessive extent (agonistic effect).
- They may bind to the receptor but not activate it. Instead the presence of the chemical on the receptor will prevent binding of the natural hormone (antagonistic effect).
- They may bind to transport proteins in the blood, thus altering the amounts of natural hormones that are present in the circulation.
- They may interfere with the metabolic processes in the body, affecting the synthesis or breakdown rates of the natural hormones.

Chemicals with hormonal activity, i.e. potential endocrine disrupters, include among others, man-made chemicals and by-products released into the environment like Persistent Organic Pollutants (POPs). Even though the intended uses of pesticides, plasticizers, antimicrobials, and flame retardants are beneficial, effects on human health are a global concern. New in vitro, in vivo, and epidemiological studies link human EDC exposure with obesity, metabolic syndrome, and type 2 diabetes. In a recently published review the main chemical compounds that may contribute to metabolic disruption are given [Casals-Casas and Desvergne, 2011]. The study summarizes that Persistent Organic Pollutants such as organochlorine pesticides are suspected of metabolic disruption activity. These results are supported by Elobeid et al [2010] that the body mass index and the waist circumference are associated with POPs levels, making the chemicals plausible contributors to the obesity epidemic. The hypothesis of whether early life exposure (both pre- and early postnatal) to endocrine-disrupting chemicals (EDCs) may be a risk factor for obesity and related metabolic diseases later in life will be tested in the European research project OBELIX (OBesogenic Endocrine disrupting chemicals: LInking prenatal eXposure to the development of obesity later in life). This project started in May 2009 and its project design is published by Legler at al. [2011].

A lot of evidence is given that there is a strong relationship between the exposure of women with chemicals and the development of cryptorchidism (malformation of the testis in the male offspring). The association between congenital cryptorchidism and some persistent pesticides in breast milk as a proxy for maternal exposure suggests that testicular descent in the fetus may be adversely affected. It has been demonstrated in case-control studies that prenatal exposure to some pesticides can adversely affect male reproductive health in animals. A possible human association between maternal exposure to POPs used as pesticides and cryptorchidism among male children has been investigated. These investigations have shown striking differences in semen quality and testicular cancer rate between Denmark and Finland. Since malformation of the testis is a shared risk factor for these conditions, a joint prospective study for the prevalence of congenital cryptorchidism was executed in Denmark (1997-2001) and Finland (1997-1999). These data have already been analysed by classical statistical methods [Boisen et al, 2004], [Damgaard et al, 2006], [Shen et al, 2008]. The studies suggested an association between congenital cryptorchidism and some persistent organochlorine pesticides present in mothers' breast milk. Thus, prenatal exposure to persistent organochlorine pesticides may adversely affect testicular descent in boys.

2. Materials and Methods

2.1. Data analysis method: Hasse diagram technique

The data analysis method is the method of partially ordered sets. Partial order theory is a discipline of Discrete Mathematics and one may consider partial order theory as an example of mathematics without arithmetic. A good overview can be found in Bruggemann and Carlsen [2006] and Bruggemann and Patil [2010].

Partial order as a discipline of Discrete Mathematics will only be briefly outlined in this paper in order to support the understanding of the data analyses steps.

First step: We need a set of objects. We call this set of objects the ground set, and denote it as G. Objects can be chemicals, strategies (for example: water management), geographical units, environmental and chemical databases, etc.

Second step: We need an operation between any two objects. As an evaluation is our aim, we must compare the objects. Is object "a" better than object "b"? If objects a and b are comparable we write $a \perp b$, albeit this in general important symbol is actually not needed in the analysis.

Third step: We do not only want that two objects are comparable, but we also would like to know the orientation: Is "a" better or worse than "b"? Therefore the signs $\leq$ and $\geq$ are introduced: $a \leq b$ "may" denote that a is better than b, $a \geq b$ "may" indicate that a is worse b.

Fourth step: Why "may"? The essential point is that we have to define, when we will consider object a as better than b, i.e. the signs "$\leq$" and "$\geq$" alone do not help in an evaluation procedure, we must give them an appropriate sense, i.e. an appropriate orientation. If objects are not comparable this is indicated by the symbol $\|$. A subset $G' \subseteq G$ in which for all object pairs $(x,y) \in G'^2$ $x \| y$ holds, is called an antichain.

Fifth step: Independent of how we define $\leq$ and $\geq$, the ground set equipped with e.g. "$\leq$" must obey three axioms, if we want to speak of a partially ordered set (poset):

Reflexivity: An object a can be compared with itself: $a \leq a$

Antisymmetry: If a is better b, and at the same time b is better than a, then a = b. We write:

$a \leq b$ and $b \leq a \Rightarrow a \cong b$. Later we will relax this axiom.

Transitivity: If a is better than b and at the same time b is better than c, then a is better than c.

$a \leq b, b \leq c \Rightarrow a \leq c$.

If the $\leq$-relation is defined properly; the ground set G equipped with $\leq$ is a partially ordered set. A widespread notation is: $(G, \leq)$.

Sixth step: Why can a partially ordered set be represented by a directed graph? Consider the objects of a ground set as vertices. Then in any case, where for $(a,b) \in G^2$ is valid $a \leq b$ we draw an arrow starting from b and ending in a. Because of the transitivity we can omit such arrows, which are represented by a sequence of $\leq$ - relations. Hence, most of the advices how to construct a Hasse diagram are using the concept of a "cover relation” $\prec$: Two objects a,b for which is valid that a ≤ b are in a cover relation, if there is no third element in between. Then a Hasse diagram is a graph of cover relations with additional conventions how to locate the objects in the drawing plane.

2.2. Software package PyHasse and the Similarity module

The new and innovative software package is that of PyHasse, written by the second author Dr. Rainer Bruggemann is under constant development. Python is used as ‘rapid prototyping’ programming language. Python belongs to the so-called ‘Very High Level Languages’ (VHLL) which allows a high level of abstraction [Muller and Schwarzer, 2007]. The concept of ‘dictionaries’ by which complex relations can efficiently be programmed may be given as an example. Python can freely be downloaded, for instance from http://www.python.org/download, is platform independent and runs on many operating systems. Python has access to many packages either in algebra, in statistics, in graphics, or cloud computing, or even in preparing sound effects, game developments and image processing. Python allows object-oriented programming and is itself modularized to a very high degree. On http://www.pythonology.com/home one finds many more pieces of information. In contrast to WHASSE PyHasse is considered as "experimental" software, which is considered as to bridge the gap between professional software and software, exclusively developed in laboratories and in general only applicable by the developer.

One important module is that of the calculation of the similarity of two data sets respectively two Hasse diagrams. This PyHasse feature gives the user quantitative information about the similarity of posets of two data sets. The mathematical background can be found e.g. in Voigt et al. [2010] where this feature is applied on environmental health data. In the similarity analysis we intend to calculate the similarity of different posets (partially ordered sets).

We distinguish among isotone, antitone, weak isotone, indifferent and identical relations. Most important are the entries like >> or <<, which are counting the "isotone" character of both partial orders and the entries like ><, <> which contribute to the "antitone" character, i.e. to the conflicts between the two partial orders. Following Rademaker et al. [2008]: two posets are in conflict or "contradict each other" (are antitone) on two objects x, y $\in$ P ,”if we have $x <_1 y$ and $y <_2 x$ or $y <_1 x$ and $x <_2 y$”. There are still more combinations to look upon: <||, >||, ||>,||< , =||, ||= or || || are considered as indifferent, combinations like > =, < =, = <, = > are called weak isotone. Finally the entry of type = = contributes to equivalence relations. Note that we use the equality sign “=”instead of the sign indicating equivalence, $\cong$, for the sake of simplicity.

2.3. Data set of 20 POPs

As mentioned above, studies were performed in Denmark (1997-2001) and Finland (1997-1999) on the occurrence of POPs in breast milk samples.

In our data analysis approach we investigated data sets of breast milk samples of women in Denmark and Finland which contained measurable levels of 20 Persistent Organic Pollutants (POPs). In this study we investigated those 20 POPs which are also found in the Stockholm’s convention. The data set differs slightly by 3 chemicals to those data set which has already been published [Voigt et al, 2010] which comprises those chemicals with complete data.

The question arises whether the above mentioned methodology of partial orders can detect differences between or similarities between these two countries. Therefore we have to evaluate a data matrix with 20 rows (the chemicals) and 65 columns (the breast milk samples of 65 anonymous women in Denmark and Finland).

In Table 1 we list the 20 POPs with their abbreviation and CAS-numbers. These abbreviations are used in the Hasse diagrams.

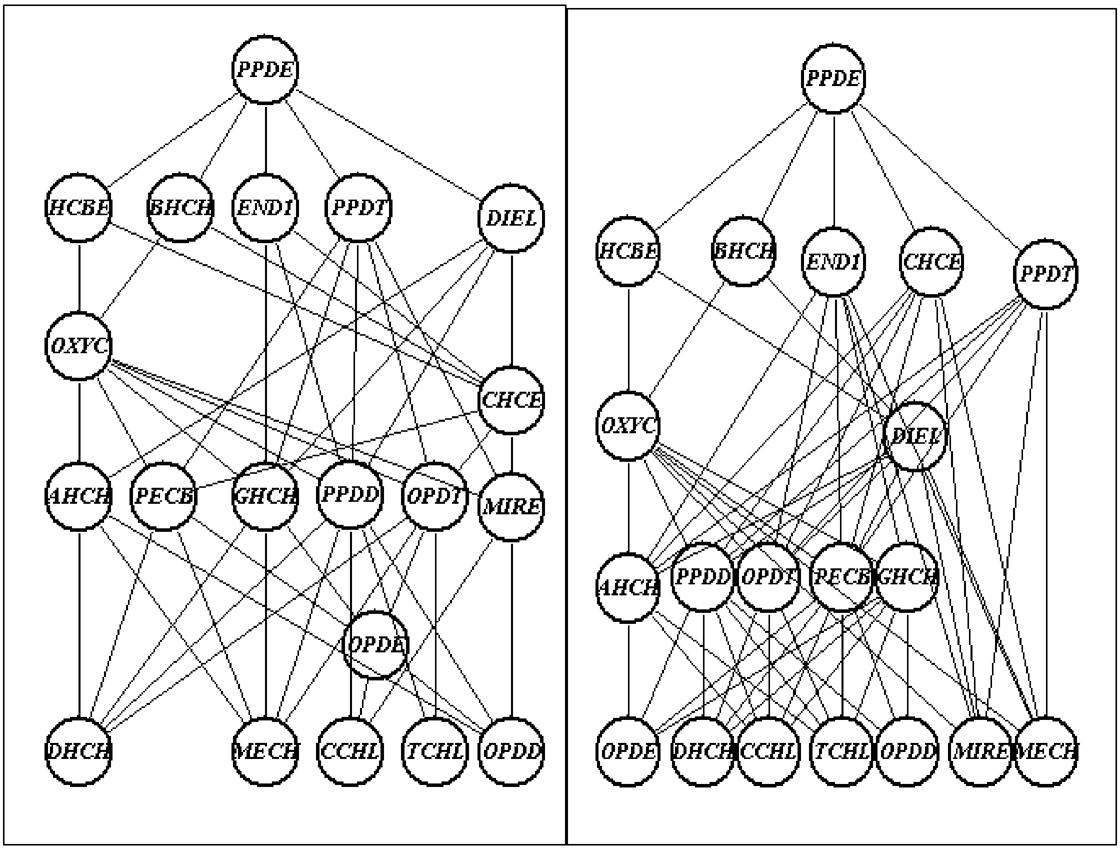
Table 1: List of 20 chlorinated POPs in Denmark, Finland

Nr.	**Acronym**	**Name**	**CAS-Number**
01	**AHCH**	alpha-Hexachlorcyclohexane	319-84-6
02	**BHCH**	beta-Hexachlorcyclohexane	319-85-7
03	**CCHL**	cis-Chlordane	5103-71-9
04	**CHCE**	cis-Heptachloroepoxide	1024-57-3
05	**DHCH**	delta-Hexachlorcyclohexane	319-86-8
06	**DIEL**	Dieldrin	60-57-1
07	**END1**	Endosulfan-1	959-98-8
08	**GHCH**	gamma-Hexachlorcyclohexane	58-89-9
09	**HCBE**	Hexachlorobenzene	118-74-1
10	**MECH**	Methoxychlor	72-43-5
11	**MIRE**	Mirex	2385-85-5
12	**OPDD**	o, p'-Dichlordiphenyldichlorethane	53-19-0
13	**OPDE**	o, p'-Dichlordiphenyldichlorethene	3424-82-6
14	**OPDT**	o, p'-Dichlordiphenyltrichlorethane	789-02-6
15	**OXYC**	Oxychlordane	27304-13-8
16	**PECB**	Pentachlorobenzene	608-93-5
17	**PPDD**	p, p'-Dichlordiphenyldichlorethane	72-54-8
18	**PPDE**	p, p'-Dichlordiphenyldichlorethene	72-55-9
19	**PPDT**	p, p'-Dichlordiphenyltrichlorethane	50-29-3
20	**TCHL**	trans-Chlordane	5103-74-2

3. Data Evaluation by the Hasse Diagram Technique

3.1. Hasse diagrams for Danish and Finnish data sets

In Figure 1 we demonstrate the results of the calculation of the Hasse diagram technique for Denmark and Finland.



Denmark ***Finland***

Figure 1: Hasse diagrams for 20 chemicals in breast milk samples in Denmark (lhs), Finland (rhs)

From the Hasse diagrams one can easily see that the chemical PPDE is always a maximal object. As we only encounter one maximal object we also speak of the greatest object. This means that it is the most dangerous pollutant in comparison with all other chemicals with which it is connected by lines in the downward direction. PPDE is the first degradation product of DDT (PPDT). DDT is always found in the second level directly connected to DDE.

With respect to the minimal objects we find the POPs CCHL, DHCD, MECH, OPDD, and TCHL in both diagrams in this position. In Finland two more minimal objects are shown, namely OPDE and MIRE. They are the least pollutants in these chosen 20 chemicals encompassing data sets. Whereas the Hasse diagram representing the Danish data shows 6 levels, the Finnish data gives 5 levels.

3.2. Similarity analysis applied on Danish and Finnish data sets

The quantification of the similarity of two Hasse diagrams is now performed applying the PyHasse software tool Similarity. On the left hand side you can see the PyHasse screen for this module and on the right hand side the results for the two data sets to be compared.

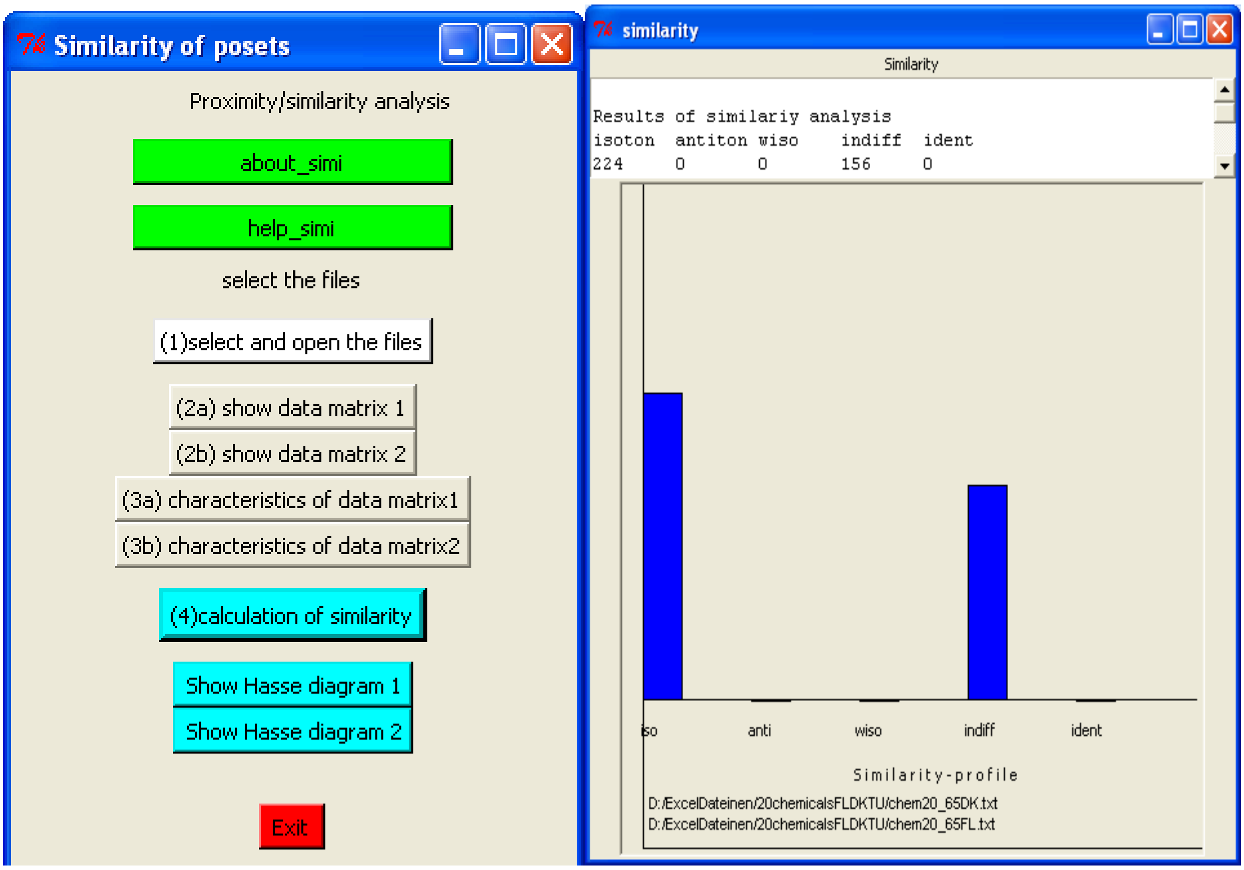


Figure 2: Similarity Analysis Tool and its Application on Danish and Finnish data

Only isotone (224) and indifferent (156) relations are calculated. The high amount of isotone relations reflects the great similarity between these two data sets, whereas the indifferent relations indicate combinations of comparabilities of the one Hasse diagram with incomparabilities of the other one. As more isotone relations are calculated than indifferent ones we can state a high degree of similarity of both data sets. This means that the 20 chosen POPs, 18 of which are found in the Stockholm Convention list on POPs (UNEP, 2011) are not only merely detected in breast milk samples both in Denmark as well as in Finland but are also found in very similar positions in the Hasse diagrams.

4. Discussion of Results

The first degradation product of DDT, DDE is always the most important pollutant concerning the contamination of breast milk samples in Denmark and Finland. One of the possible reasons for that is that the production of DDT is banned in many countries. In the 1970s and 1980s, agricultural use was banned in most developed countries, beginning with Hungary in 1968, then in Norway and Sweden 1970, in Germany and the United States in 1972, but not in the United Kingdom until 1984. Production for vector control use has not been banned. The production figures vary have not been published worldwide. In 1960 the United States produces 74,600 t, in 1970 only 26,900 t. Germany had a production figure of 30,000 t in 1965. [WIKIPEDIA, 2011]. The production figures in Russia were about 15,000 – 25,000 t, in Italy 10,000t /a in the sixties. In the European countries 9,500 t were produced in 1981. Based on response from the questionnaire sent out by UNEP and from reports from the Global Environment Facility, for 2005, the total production globally for DDT for vector control is estimated at 6,269t [UNEP, 2007]. In a recently published paper the production figures were around 4,740 t/2005 worldwide production. DDT is currently being produced in three countries: India, China, and the Democratic People's Republic of Korea [van den Berg, 2009]. The slowdown in the production and hence application of DDT is demonstrated in the Hasse diagrams of the Danish and Finnish breast milk samples as the DDE, one of the first degradation products of DDT in found in the maximal position and not the DDT itself.

The situation of Lindane (GHCH), the gamma isomer of Hexachlorocyclohexane is comparable to those of DDT. The byproducts (which are more toxic than the initial product) of the Lindane production the beta isomer (BHCH) is found in a higher position than the active ingredient. The alpha isomer (AHCH) is situated in the same level as Lindane. This chemical is put on the second list of POPs in 2009. A global perspective on the management of Lindane and its waste isomers is given by Vijgen et al. [2011]. In a preliminary assessment, the countries and the respective amount of HCH residues stored and deposited from Lindane production are estimated. Between 4 and 7 million tons of wastes of toxic, persistent and bioaccumulative residues (largely consisting of alpha- (approx. 80%) and beta- HCH) are estimated to have been produced and discarded around the globe during 60 years of Lindane production. For approximately 1.9 million tonnes, information is available regarding deposition. It can be expected that most locations where HCH waste was discarded/stockpiled are not secured and that critical environmental impacts are resulting from leaching and volatilisation. Production and agricultural use is banned the 169 countries that parties to the Stockholm Convention, but pharmaceutical use is allowed until 2015 [UNEP 2011].

Taking a look a Dieldrin (DIEL) which is situated in the second highest level in the Danish Hasse diagram and in the middle level position in the Finnish diagram, it has been linked to health problems such as e.g. Parkinson's disease. Weisskopf et al. [2010] examined the association between prospective serum biomarkers of organochlorine pesticides and Parkinson disease. The results provide some support for an increased risk of Parkinson disease with exposure to Dieldrin, but chance or exposure correlation with other less persistent pesticides could contribute to their findings as well.

Concerning the similarity analysis it can be stated that in both countries the breast milk samples are similarily polluted with POPs.

5. Conclusion and Outlook

Environmetrical/Chemometrical methods play an essential part in supporting the urgent need for coping with environmental health topics worldwide. One of the imminent topics is those of Persistent Organic Pollutants. The implementation of the Stockholm Convention on Persistent Organic Pollutants [UNEP, 2011] is one extremely important application field worldwide. The introduced Hasse diagram technique with its software package PyHasse can be applied for supporting the exact compliance with the outlaw of POPs. We conclude that the data analysis method presented here, can well be applied for distinguishing more or less dangerous chemicals. In addition, it should be used in sustainable chemistry in the same manner for detecting more and less sustainable chemicals.

Several more features of the data analysis method of partially ordered sets, e.g. the sensitivity analysis (finding out the most important attributes), the separability analysis (separability of two sub sets of data), can be applied for receiving a deeper knowledge about environmental health data on POPs. Furthermore, we intend to cooperate with Turkish colleagues who performed environmental health studies on Persistent Organic Pollutants in the Taurus Mountains in Turkey.

The vast field of implications of chemical wastes of POPs has to be coped with in the near future. Apart from the chemical waste, the other two large worldwide waste problems, namely nuclear wastes and greenhouse gases have to be tackled as well with the same priority. Society is facing huge duties and responsibilities with respect to environmental and human health and Environmetrics and Chemometrics, applied systematically and effectively could be of great support.

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