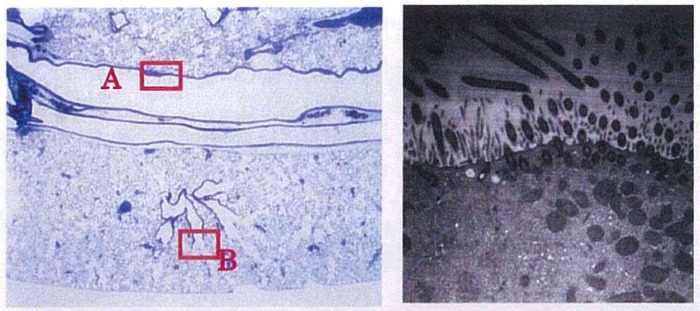
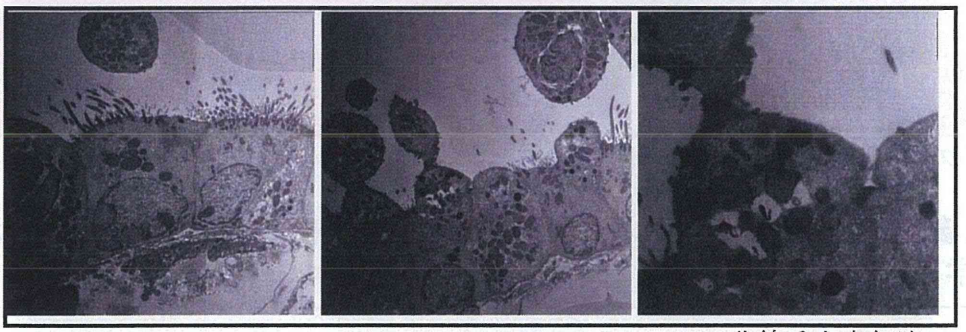
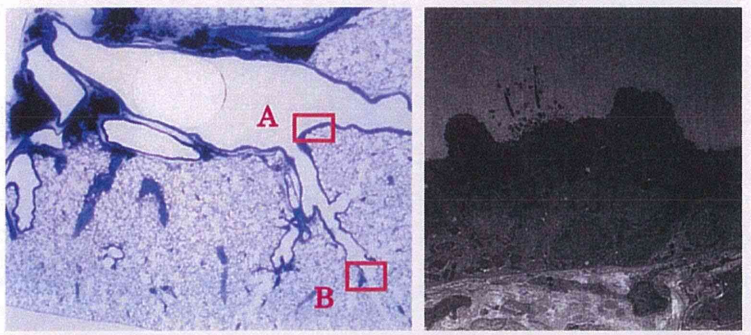


A: 線毛上皮細胞

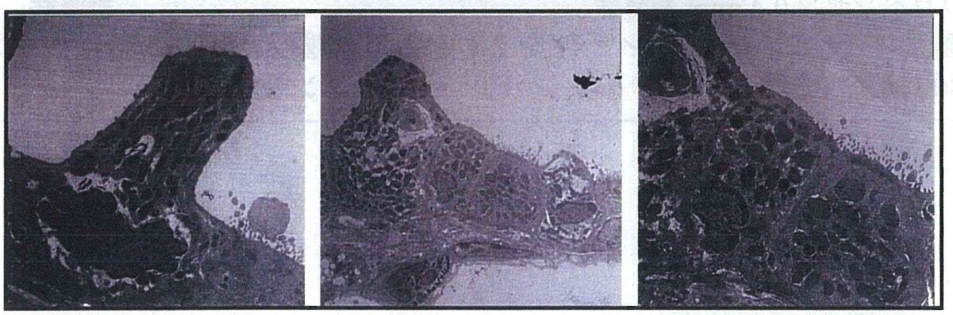


B: 非線毛上皮細胞

図 9 極低濃度キシレン 28 日間暴露試験、2.0 ppm 暴露群（動物番号:301）

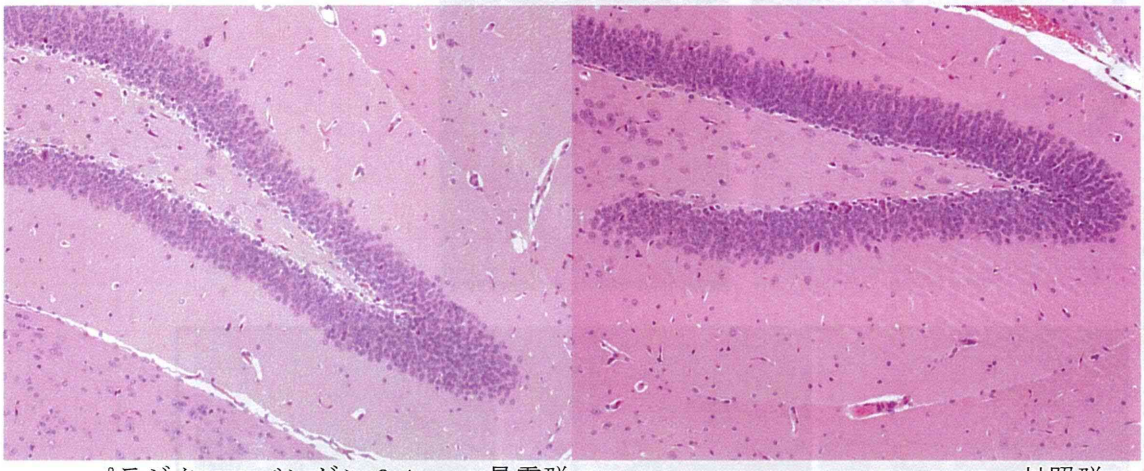


A: 線毛上皮細胞



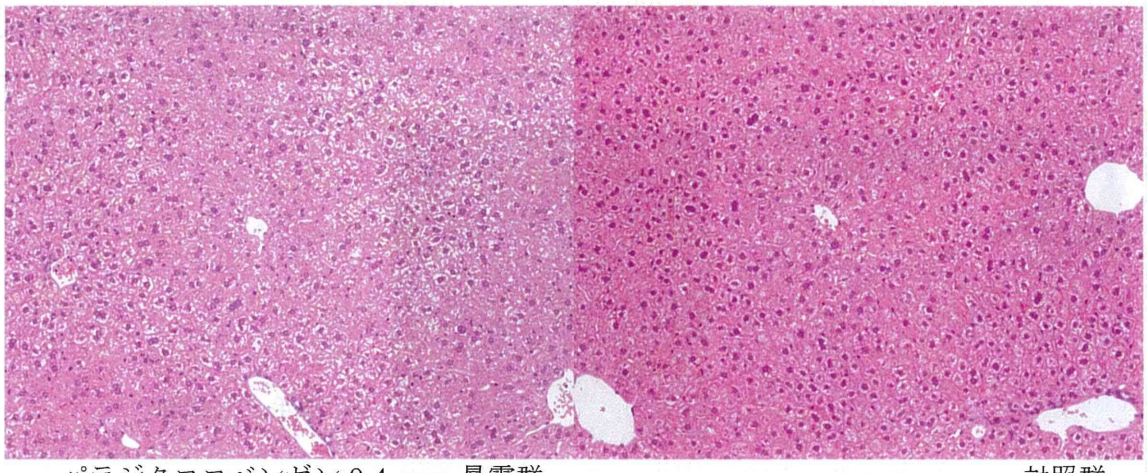
B: 非線毛上皮細胞

図 10 極低濃度キシレン 28 日間暴露試験、2.0 ppm 暴露群（動物番号:302）



パラジクロロベンゼン 0.4 ppm 暴露群　　対照群

図 11 極低濃度パラジクロロベンゼンを 28 日間反復吸入暴露したマウスの病理組織学的検査、脳（海馬）、ヘマトキシリン・エオジン染色



パラジクロロベンゼン 0.4 ppm 暴露群　　対照群

図 12 極低濃度パラジクロロベンゼンを 28 日間反復吸入暴露したマウスの病理組織学的検査、肝臓、ヘマトキシリン・エオジン染色

表1 実測暴露濃度（パラジクロロベンゼン:22時間/日×28日間）

Day	Exp. Group			
	control	0.04 ppm	0.12 ppm	0.4 ppm
1	0.000	0.041	0.127	0.408
2	0.000	0.052	0.164	0.536
3	0.000	0.044	0.134	0.443
4	0.000	0.040	0.124	0.413
5	0.000	0.057	0.187	0.636
6	0.000	0.056	0.187	0.626
7	0.000	0.049	0.162	0.629
8	0.000	0.040	0.144	0.581
9	0.000	0.026	0.100	0.401
10	0.000	0.048	0.167	0.556
11	0.000	0.045	0.142	0.500
12	0.000	0.054	0.172	0.561
13	0.000	0.038	0.123	0.394
14	0.000	0.038	0.113	0.323
15	0.000	0.033	0.112	0.344
16	0.000	0.036	0.117	0.350
17	0.000	0.040	0.115	0.343
18	0.000	0.032	0.108	0.292
19	0.000	0.028	0.095	0.297
20	0.000	0.026	0.086	0.264
21	0.000	0.039	0.118	0.400
22	0.000	0.035	0.104	0.373
23	0.000	0.028	0.085	0.303
24	0.000	0.023	0.084	0.300
25	0.000	0.036	0.115	0.435
26	0.000	0.031	0.106	0.391
27	0.000	0.029	0.100	0.366
28	0.000	0.030	0.089	0.314
Average	0.000	0.038	0.124	0.421
S.D.	0	0.009467747	0.030551364	0.113285312

表2 体重推移 (パラジクロロベンゼン:22時間/日、28日間)

Exp. Group	Nomber of animals	Exposure 0	4	7	(day)
Control	6	23.6 ± 0.5	23.6 ± 0.8	24.3 ± 0.9	
0.04 ppm	6	23.6 ± 0.5	23.4 ± 1.1	23.5 ± 1.2	
0.12 ppm	6	23.6 ± 0.5	23.4 ± 0.7	23.9 ± 0.8	
0.4 ppm	6	23.9 ± 0.6	23.8 ± 0.7	24.2 ± 0.7	

Exp. Group	Nomber of animals	Exposure 11	13	18	(day)
Control	6	24.1 ± 0.8	24.3 ± 0.6	24.8 ± 0.7	
0.04 ppm	6	23.6 ± 1.2	23.8 ± 0.6	24.2 ± 1.1	
0.12 ppm	6	24.0 ± 0.6	24.4 ± 0.8	24.9 ± 1.0	
0.4 ppm	6	24.0 ± 0.7	24.6 ± 0.4	25.1 ± 0.7	

Exp. Group	Nomber of animals	Exposure 21	25	28	(day)
Control	6	24.9 ± 0.6	25.5 ± 0.4	26.1 ± 0.8	
0.04 ppm	6	24.3 ± 1.4	25.0 ± 1.7	25.1 ± 1.8	
0.12 ppm	6	25.3 ± 0.8	25.4 ± 0.8	26.0 ± 0.9	
0.4 ppm	6	25.2 ± 0.8	25.6 ± 0.8	26.2 ± 0.7	

平均値±標準偏差 *P<0.05,**P<0.01)

表3 摂餌量推移 (パラジクロロベンゼン:22時間/日、28日間)

Exp. Group	Number of animals	Exposure 4	7	11	(day)
Control	6	4.5 ± 0.3	4.3 ± 0.2	3.2 ± 0.2	
0.2 ppm	6	4.5 ± 0.3	4.1 ± 0.3	3.3 ± 0.1	
0.7 ppm	6	4.5 ± 0.3	4.3 ± 0.3	3.6 ± 0.2	
2.0 ppm	6	4.7 ± 0.2	4.5 ± 0.2	3.5 ± 0.2	

Exp. Group	Number of animals	Exposure 13	18	21	(day)
Control	6	3.3 ± 0.4	3.5 ± 0.3	3.6 ± 0.3	
0.2 ppm	6	3.4 ± 0.2	3.6 ± 0.3	3.6 ± 0.4	
0.7 ppm	6	3.8 ± 0.3	3.8 ± 0.3	4.1 ± 0.3	
2.0 ppm	6	3.8 ± 0.3	3.9 ± 0.3	4.0 ± 0.3	

Exp. Group	Number of animals	Exposure 25	28	(day)
Control	6	3.2 ± 0.1	3.5 ± 0.3	
0.2 ppm	6	3.2 ± 0.2	3.1 ± 0.2	
0.7 ppm	6	3.3 ± 0.2	3.6 ± 0.5	
2.0 ppm	6	3.4 ± 0.1	3.5 ± 0.3	

平均値±標準偏差 *P<0.05,**P<0.01)

表4 各臓器の絶対・相対重量及び最終体重（パラジクロロベンゼン:22時間×28日間）

肝

Exp. Group	Liver (g/100g)	Liver (g)	Body weight (g)
Air control	5.463 ± 0.282	1.426 ± 0.085	26.1 ± 0.8
0.04 ppm	5.374 ± 0.112	1.351 ± 0.106	25.1 ± 1.8
0.12 ppm	5.575 ± 0.110	1.450 ± 0.070	26.0 ± 0.9
0.4 ppm	5.382 ± 0.544	1.411 ± 0.165	26.2 ± 0.7

腎

Exp. Group	Kidny (g/100g)	Kidny (g)	Body weight (g)
Air control	1.077 ± 0.074	0.281 ± 0.017	26.1 ± 0.8
0.04 ppm	1.125 ± 0.053	0.282 ± 0.018	25.1 ± 1.8
0.12 ppm	1.104 ± 0.034	0.287 ± 0.015	26.0 ± 0.9
0.4 ppm	1.159 ± 0.061	0.303 ± 0.013	26.2 ± 0.7

脾

Exp. Group	Spleen (g/100g)	Spleen (g)	Body weight (g)
Air control	0.245 ± 0.021	0.064 ± 0.007	26.1 ± 0.8
0.04 ppm	0.251 ± 0.030	0.064 ± 0.011	25.1 ± 1.8
0.12 ppm	0.255 ± 0.016	0.066 ± 0.004	26.0 ± 0.9
0.4 ppm	0.276 ± 0.026	0.072 ± 0.006	26.2 ± 0.7

心

Exp. Group	Heart (g/100g)	Heart (g)	Body weight (g)
Air control	0.496 ± 0.029	0.129 ± 0.005	26.1 ± 0.8
0.04 ppm	0.517 ± 0.032	0.130 ± 0.007	25.1 ± 1.8
0.12 ppm	0.520 ± 0.049	0.135 ± 0.011	26.0 ± 0.9
0.4 ppm	0.506 ± 0.034	0.132 ± 0.008	26.2 ± 0.7

脳

Exp. Group	Brain (g/100g)	Brain (g)	Body weight (g)
Air control	1.708 ± 0.095	0.445 ± 0.013	26.1 ± 0.8
0.04 ppm	1.790 ± 0.050	0.449 ± 0.019	25.1 ± 1.8
0.12 ppm	1.732 ± 0.057	0.450 ± 0.007	26.0 ± 0.9
0.4 ppm	1.718 ± 0.085	0.450 ± 0.016	26.2 ± 0.7

胸腺

Exp. Group	Thymus (g/100g)	Thymus (g)	Body weight (g)
Air control	0.176 ± 0.026	0.046 ± 0.006	26.1 ± 0.8
0.04 ppm	0.182 ± 0.039	0.046 ± 0.011	25.1 ± 1.8
0.12 ppm	0.190 ± 0.019	0.050 ± 0.005	26.0 ± 0.9
0.4 ppm	0.169 ± 0.017	0.044 ± 0.003	26.2 ± 0.7

平均値±標準偏差 *P<0.05,**P<0.01）

表 5　病理組織学的検査結果

濃度	0 ppm	0.04 ppm 群	0.12 ppm 群	0.40 ppm 群
検索数	6	6	6	6
肝臓	炎症性細胞集簇巣 1+:3	炎症性細胞集簇巣 1+:4 巣状壊死 1+:1	炎症性細胞集簇巣 1+:5	炎症性細胞集簇巣 1+:2
腎臓	著変なし	著変なし	著変なし	著変なし
脾臓	著変なし	著変なし	著変なし	著変なし
心臓	著変なし	著変なし	著変なし	著変なし
脳	著変なし	著変なし	著変なし	著変なし

1+:軽度

病理組織学的検査で各臓器にキシレンの暴露による影響を認めなかった.

別添５

Ⅲ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻名	ページ	出版年
Kanno J, Aisaki K, Igarashi K, Kitajima S, Matsuda N, Morita K, Tsuji M, Moriyama N, Furukawa Y, Otsuka M, Tachihara E, Nakatsu N, Kodama Y	Oral administration of pentachlorophenol induces interferon signaling mRNAs in C57BL/6 male mouse liver.	J Toxicol Sci.	38 (4)	643 - 654	2013
Taquahashi Y, Ogawa Y, Takagi A, Tsuji M, Morita K, Kanno J	An improved dispersion method of multi-wall carbon nanotube for inhalation toxicity studies of experimental animals.	J Toxicol Sci.	38 (4)	619 - 628	2013
Noguchi S, Hamano E, Matsushita I, Hijikata M, Ito H, Nagase T, Keicho N.	Differential effects of a common splice site polymorphism on the generation of OAS1 variants in human bronchial epithelial cells.	Hum Immunol	74 (3)	395 - 401	2013
Noguchi S, Hijikata M, Hamano E, Matsushita I, Ito H, Ohashi J, Nagase T, Keicho N.	MxA transcripts with distinct first exons and modulation of gene expression levels by single-nucleotide polymorphisms in human bronchial epithelial cells.	Immunogenetics	65 (2)	107 - 114	2013

Ohnishi M, Umeda Y, Katagiri T, Kasai T, Ikawa N, Nishizawa T, Fukushima S.	Inhalation carcinogenicity of 1,1,1-trichloroethane in rats and mice.	Inhalation Toxicology	25	298 - 306	2013
Ohnishi M, Yajima H, Kasai T, Umeda Y, Yamamoto M, Yamamoto S, Okuda H, Suzuki M, Nishizawa T, Fukushima S.	Novel method using hybrid markers: development of an approach for pulmonary measurement of multi-walled carbon nanotubes.	Journal of Occupational Medicine and Toxicology	8 30		2013

別添 6

Ⅳ. 研究成果の刊行物・別刷

The Journal of Toxicological Sciences (J. Toxicol. Sci.)
Vol.38, No.4, 643-654, 2013

Original Article

Oral administration of pentachlorophenol induces interferon signaling mRNAs in C57BL/6 male mouse liver

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(Received June 17, 2013; Accepted June 25, 2013)

ABSTRACT — Pentachlorophenol (PCP) was monitored for transcriptome responses in adult mouse liver at 2, 4, 8 and 24 hr after a single oral administration at four dose levels, 0, 10, 30 and 100 mg/kg. The expression data obtained using Affymetrix GeneChip MOE430 2.0 were absolutized by the Percellome method and expressed as three dimensional (3D) surface graphs with axes of time, dose and copy numbers of mRNA per cell. We developed the programs RSort, for comprehensive screening of the 3D surface data and PercellomeExploror for cross-referencing and confirmed the significant responses by visual inspection. In the first 8 hr, approximately 100 probe sets (PSs) related to PXR/SXR and Cyp2a4 and other metabolic enzymes were induced whereas Fos and JunB were suppressed. At 24 hr, about 1,200 PSs were strongly induced. We cross-referenced the Percellome database consisting of 111 chemicals on the liver transcriptome and found that about half of the PSs belonged to the metabolic pathways including Nrf2-mediated oxidative stress response networks shared with some of the 111 chemicals. The other half of the induced genes were interferon signaling network genes (ISG) and their induction was unique to PCP. Toll like receptors and other pattern recognition receptors, interferon regulatory factors and interferon alpha itself were included but inflammatory cytokines were not induced. In summary, these data indicated that functional symptoms of PCP treatment, such as hyperthermia and profuse sweating might be mediated by the ISG rather than the previously documented mitochondrial uncoupling mechanism. PCP might become a hint for developing low molecular weight orally available interferon mimetic drugs following imiquimod and RO4948191 as agonists of toll-like receptor and interferon receptor.

Key words: Pentachlorophenol, Mouse, Liver, Interferon signaling genes, Percellome toxicogenomics

INTRODUCTION

The Percellome Toxicogenomics Project is designed to identify dynamic and extensive networks of genes whose time- and dose-dependent patterns of expression in response to a chemical allows its toxic effects to be predicted. For this project, we developed a standardization method for microarrays and quantitative PCR that produces copy number of mRNAs per one cell (designated as "Percellome method") (Kanno *et al.*, 2006). This method allowed us to directly and quantitatively compare gene expression data among samples, studies, organs and even species using four arithmetic operations. One hundred and eleven chemicals (as of June 2013, Supplementary Table 1), most of which are known for their toxicity, were examined using the standard protocol of the project.

Pentachlorophenol (PCP) was examined in adult male C57BL/6 mouse liver. This compound has been used for multiple purposes such as herbicide, insecticide, fungicide, disinfectant, and other preservative purposes, moreover, its metabolism and toxicity, including carcinogenicity have been well studied. PCP is known to induce morphological changes in liver, kidney, hematopoietic, respiratory, immune and neural systems together with irritation of exposed sites. Hepatocarcinogenicity was demonstrated in rodents; the postulated mechanism involves

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hydroxyl radical mediated DNA adduct formation and oxidative stress by the PCP metabolites. Functional symptoms, such as hyperthermia (sometimes life-threatening), profuse sweating, nausea, and uncoordinated movements were noted. Hyperthermia and other functional symptoms have been explained by the uncoupling of oxidative phosphorylation in mitochondria.

Here, we report that a comprehensive Percellome analysis revealed that PCP was the only chemical among the 111 tested in our project that strongly induced the interferon signaling gene (ISG) network. Additional pathways induced by PCP were Nrf2-mediated oxidative stress responses and other metabolic pathways more commonly seem among the 111 chemicals.

MATERIALS AND METHODS

Test chemical

PCP, standard grade (100.0% by gas chromatography coupled with flame ionization detector, Wako Pure Chemical Industries, Ltd., Tokyo, Japan) was dissolved in water containing 0.5% methyl cellulose (Shin-Etsu Chemical Co., Ltd., Tokyo, Japan).

Animal experiments

All experiments were carried out under approval of Experimental Animal Use Committee of National Institute of Health Sciences, Japan. C57BL/6 Cr Slc (Japan SLC, Inc., Shizuoka, Japan) twelve week-old male mice maintained in a barrier system with a 12 hr photoperiod were used in this study. Prior to the main study, a dose finding study was performed. This study revealed that 100 mg/kg was the maximum dose without clinical symptoms or alteration in H&E histology of the liver sampled 24 hr after single oral administration (a standard criteria for the top dose of the Percellome Project study). For the liver transcriptome experiments, forty eight mice were divided into four groups with twelve each, and given a single dose of PCP at 0, 10, 30 and 100 mg/kg by oral gavage. At 2, 4, 8 and 24 hr post-gavage, three randomly selected mice from each dose groups were euthanized by exsanguination under ether anesthesia and the livers were excised into ice-cooled plastic dishes. Tissue blocks weighing 30 to 60 mg were placed in an RNase-free 2 ml plastic tube (Eppendorf GmbH., Hamburg, Germany) and soaked in RNAlater (Ambion Inc., Austin, TX, USA) within 3 min of the beginning of anesthesia. The 12 animal sampling for each time point was finished within 25 to 30 min in order to avoid circadian-based variation within a time point.

Sample preparation and GeneChip measurement

The tissue blocks soaked in RNAlater were kept overnight at 4°C or until use. RNAlater was replaced in the 2 ml plastic tube with 1.0 ml of RLT buffer (Qiagen GmbH., Hilden, Germany), and the tissue was homogenized by adding a 5 mm diameter Zirconium bead (Funakoshi, Tokyo, Japan) and shaking with a MixerMill 300 (Qiagen GmbH) at a speed of 20Hz for 5 min (only the outermost row of the shaker box was used).

Three separate 10μl aliquots were taken from each sample homogenate to another tube and mixed thoroughly. A final 10 μl aliquot there from was treated with DNAse-free RNase A (Nippon Gene Inc., Tokyo, Japan) for 30 min at 37°C, followed by Proteinase K (Roche Diagnostics GmbH., Mannheim, Germany) for 3 hr at 55°C in 1.5 ml capped tubes. The aliquot was transferred to a 96-well black plate. PicoGreen fluorescent dye (Molecular Probes Inc., Eugene, OR, USA) was added to each well, shaken for 10 sec four times and then incubated for 2 min at 30°C. The DNA concentration was measured using a 96 well fluorescence plate reader with excitation at 485 nm and emission at 538 nm. λ phage DNA (PicoGreen Kit, Molecular Probes Inc.) was used as standard.

As reported previously, the graded-dose spike cocktail (GSC) made of the following five *Bacillus subtilis* RNA sequences were selected from the gene list of Affymetrix GeneChip arrays (AFFX-ThrX-3_at, AFFX-LysX-3_at, AFFX-PheX-3_at, AFFX-DapX-3_at, and AFFX-TrpnX-3_at) present in the MOE430 arrays was added to the sample homogenates in proportion to their DNA concentrations (Kanno *et al.*, 2006). Then, the sample homogenates spiked with GSC were processed according to the Affymetrix standard protocol. The GeneChips used were Mouse 430 2.0. We used the in house developed SCal4 (Spike Calculation version 4, by K.A.) to check the efficiency of *in vitro* transcription, and the dose-response linearity of the five GSC spikes and to produce Percellome data, i.e. absolutized mRNA copy numbers of each PS were generated.

The data consist of four dose levels and four time points, generating a 4 x 4 matrix. The mean value (m) with standard deviation (sd) was calculated from the triplicates for all of the probe sets (PSs) for each dose-time points. In order to better visualize the changes at 2 hr, the vehicle value was used for putative zero point, and drawn a 5 x 4 surface three-dimension (3D) surface graph with X-axis for dose, Y for time, and Z for expression as shown in Fig. 1.

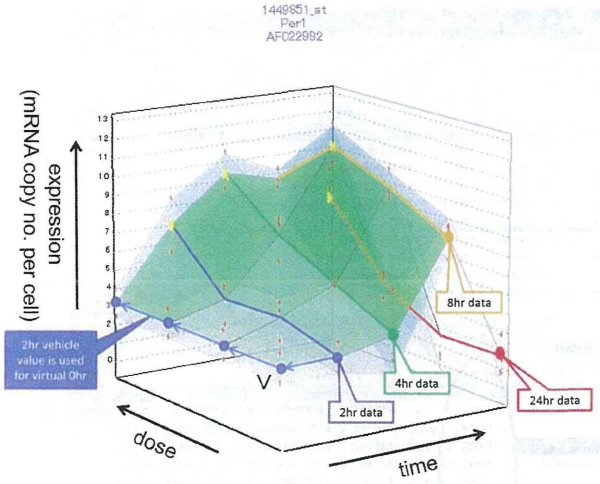

Fig. 1. Three dimensional surface expressions of Percellome Project data: The project data consist of four dose levels and four time points, generating 4 x 4 matrix. The mean value and standard deviation were calculated from the triplicate data. In order to better visualize the changes at 2 hr, the vehicle value was used for zero hour data to draw a 5 x 4 surface graph with X-axis for dose, Y for time, and Z for expression. Here, Affymetrix ID 1449851_at (Per1, period homolog 1) is shown. The 5 x 4 mesh made by the mean values was painted in translucent green (mean surface). The mean surface is rainbow-colored from blue, green, red to yellow according to its peak absolute values (cf. Fig. 3, Supplementary Fig. 1). Above and below the mean surface, +1sd and -1sd surfaces were overlaid using transparent blue. The dose-response curve at 2 hr, 4 hr, 8 hr and 24 hr are highlighted in blue, green, yellow and red. In a direction perpendicular to the dose-response curves, time course of each dose groups and vehicle group is indicated (not highlighted). The graph reads that the highest dose peaked at 4 hr at around 9 copies per cell; the middle dose peaked at 8 hr above 10 copies per cell. The vehicle group (V) showed the circadian change and peaked at 8 hr. The small red crosses are data of each animal sample (n = 3). Yellow asterisks indicate that the marked mean values were significantly different from concurrent vehicle value by $p < 0.05$ (Student's t-test).

Comprehensive selection of treatment-responding mRNAs

The in house developed software, RSort (Roughness Sort by K.A.) was used for automatic selection of treatment-responding mRNAs. This program sorts the PSs based on the roughness of the 3D surface. In other words, calculate the numbers of peaks (upward and downward) in a surface and sort by the number of peaks (maximum of eight peaks in 4 x 4 grid of the surface). Next, it filters the PSs by the number of peaks (normally three or less peaks) and additional parameters such as maximum expression level (normally more than one copy per cell for liver samples), p values between vehicle and top dose groups ($P < 0.05$ or $p < 0.01$). Here, a surface was selected when it had three peaks or less, the first peak in high doses (at any time) or the first peak in middle doses if its value is not significantly different from the neighboring high dose at $p < 0.01$ by Student's t-test, and the value of the peak is significantly different from that of vehicle control at $p < 0.05$ by Student's t-test. These automatically selected PSs were then visually checked for their 3D-surface shapes (to eliminate noisy data), and subdivided into those showed initial changes at 2, 4, 8, and 24 hr. A cross-referencing program named PE (Percellome Explorer, by K.A.) was used to select a list of chemicals that share PSs common to the visually confirmed list of PCP. The PE contains the gene lists automatically selected by the RSort of all data in our Percellome Project (168 datasets for liver samples, 286 for all samples), and automatically cross-refers and sorts out the chemicals sharing the same PSs (Fig. 2). The automatically selected gene lists (product sets) were visually checked to remove noise surfaces.

In Situ Hybridization

For *in situ* hybridization of Irf7 and Stat1 mRNAs, QuantiGeneViewRNA ISH Tissue Assay kit (Affymetrix, Inc., Santa Clara, CA, USA) was used. The probes were designed and synthesized by Affymetrix; regions covered were 2-1461 bases for Irf7 and 707-1710 bases for Stat1. 10% buffered formalin fixed liver tissues were dehydrated and embedded in paraffin. Tissue sections were mounted on "FRONTIER coated glass slides" (Matsunami Glass Ind., Ltd. Osaka, Japan). The slides were completely dried and stored until use. The slides were re-fixed in 10% formaldehyde for 1 hr at room temperature and washed with PBS and deparaffinized with xylene, pretreated in 1x Pretreatment Solution at 98°C for 30 min and digested with Protease QF at 40°C for 20 min. The probes were hybridized at 40°C for 2 hr and the signals were detected with Fast Red.

RESULTS

The numbers of PSs that started to change in response to PCP treatment at 2, 4, 8 and 24 hr were 98, 55, 127 and 1192 respectively (Supplementary Table 2, Supplementary Fig. 1). Chemicals or treatment in the Percellome database (Supplementary Table 1), that shared the PS list with PCP are shown in Table 1. The chemicals that shared the most with the 2 hr PS list of PCP

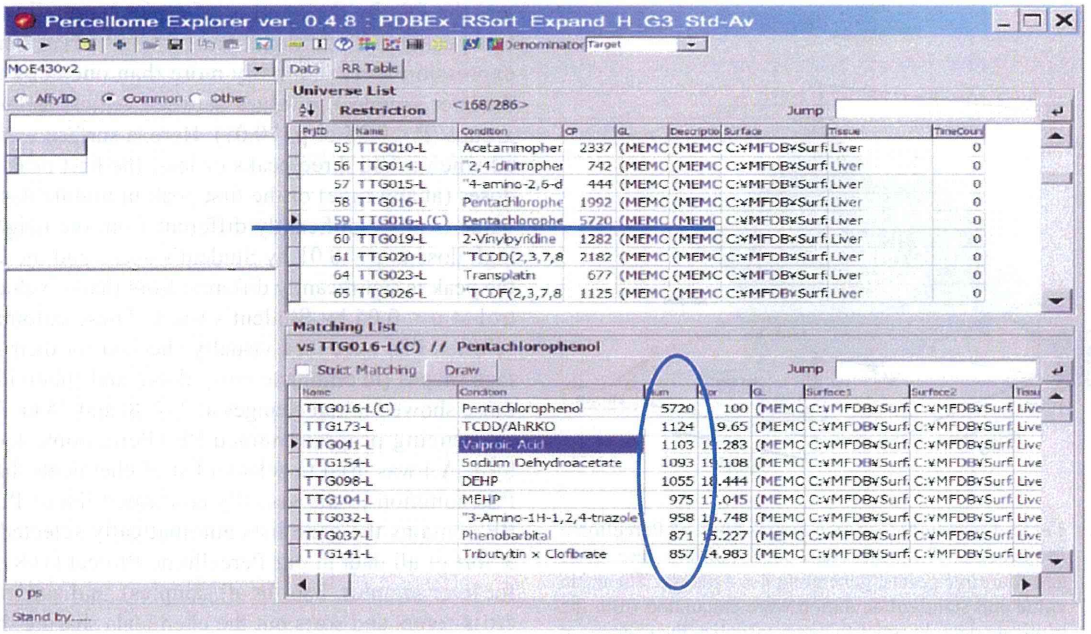

Fig. 2. PercellomeExplorer (PE) Software: The PE contains the gene lists automatically selected by the RSort software program of all data in our Percellome Project (168 datasets for liver samples, 286 for all samples, as of May 2013), and automatically picks up the chemicals sharing same PSs. TTG016-L(C), the study code for PCP was selected from the upper window and the chemicals sharing the PSs were listed in the lower window. These lists await visual confirmation.

was sodium dihydroacetate (TTG154-L); 51 PSs, followed by acephate (TTG109-L); 24 PSs, down to 5-fluorouracil (TTG160-L); 4 PSs. The sum set (or union of sets in set theory) of the 2 hr PS lists that are listed in the 2 hr column of the Table 1 contained 75 PSs (up-regulated (Up) 59, down-regulated (D) 16). Likewise, the sum set of the 4 hr PS lists contained 31 PSs (Up 22, D 9), 8 hr 46 PSs (Up 23, D 23) and 24 hr 636 PSs (all Up). The PS list unique to PCP (Unique list) at each time point contained 23, 24, 81, and 556 PSs at each time points (cf. Supplementary Table 2).

Profiles of genes changed at 2, 4 and 8 hr

The PS list common to other chemicals (Common list) contained the gluconeogenesis pathway of PGC-1A (Ppargc1a)/Foxo1/HNF4 (Puigserver *et al.*, 2003) that were induced at 2 hr (Fig. 3). This finding is in concordance with the report in experimental animals that PCP acutely induces hyperglycemia (Deichman *et al.*, 1942; Clayton and Clayton, 1981). Ppargc1a was reported to increase the expression of Lpin1 (Finck *et al.*, 2006), which was also the case here. A small set of genes encoding metabolic enzymes was induced during the first 8 hr, including Cyp2a4, Cyp4f16, Cyp7a1, Cyp17a1, Cyp39a1, Fmo2, and Fmo5 (Fig. 3).

Ingenuity pathway analysis (Ingenuity Systems, Inc. Redwood City, CA, USA) indicated that these genes are likely to be induced by Nr1i3 (CAR), Nr1i2 (PXR/SXR) or Nr5a1 (data not shown). Although our RSort program did not identify these nuclear receptors, manual search showed that PXR/SXR was induced by PCP (Fig. 3). These changes were not unique to PCP and shared by some of the chemicals in the Common list (cf. Supplementary Table 2).

Down regulation of Fos and JunB at 2, 4, and 8 hr (Fig. 3) was uniquely found in the PCP gene list. Bioinformatic analysis did not identify any associated pathways.

Profiles of genes started to change at 24 hr

The list of PSs induced at 24 hr contained two large networks. About half of the PSs showing altered expression by PCP were assigned to the interferon signaling pathway (Fig. 4, Supplementary Fig. 1). The interferon signaling genes (ISG) were highly up-regulated from Stat1, Stat2, Tyk, to Irf7, Myd88, Oas, Ifit, Cxcl10 and other downstream targets. Toll like receptors (TLRs) and

Table 1. The total numbers of probesets induced by PCP at each time points and those shared with other chemicals.

2 hr			4 hr			8 hr			24 hr		
Percellome No.	Treatment	PS	Percellome No.	Treatment	PS	Percellome No.	Treatment	PS	Percellome No.	Treatment	PS
TTG016-L(C)	Pentachlorophenol	98	TTG016-L(C)	Pentachlorophenol	55	TTG016-L(C)	Pentachlorophenol	127	TTG016-L(C)	Pentachlorophenol	1192
TTG154-L	Sodium Dehydroacetate	51	TTG104-L	MEHP	21	TTG098-L	DEHP	15	TTG098-L	DEHP	258
TTG109-L	Acephate	24	TTG098-L	DEHP	16	TTG041-L	Valproic Acid	14	TTG032-L	3-Amino-1H-1,2,4-triazole	212
TTG059-L	Caffeine	19	TTG037-L	Phenobarbital	14	TTG104-L	MEHP	14	TTG104-L	MEHP	177
TTG062-L(C)	Dexamethasone	18	TTG032-L	3-Amino-1H-1,2,4-triazole	12	TTG109-L	Acephate	13	TTG037-L	Phenobarbital	160
TTG041-L	Valproic Acid	18	TTG144-L	Tributyltin x Phenobarbital	12	TTG160-L	5-fluorouracil	10	TTG041-L	Valproic Acid	109
TTG098-L	DEHP	17	TTG150-L	Valproic acid sodium salt x Thalidomide	8	TTG154-L	Sodium Dehydroacetate	9	TTG157-L	Valproic acid sodium salt	103
TTG019-L	2-Vinylpyridine	15	TTG141-L	Tributyltin x Clofibrate	8	TTG141-L	Tributyltin x Clofibrate	8	TTG031-L	2-Chloro-4,6-dimethylaniline	94
TTG104-L	MEHP	12	TTG074-L	Bromobenzene	8	TTG031-L	2-Chloro-4,6-dimethylaniline	8	TTG154-L	Sodium Dehydroacetate	77
TTG165-L	Chlorpyrifos	12	TTG151-L	Valproic acid sodium salt x Valproic acid sodium salt	7	TTG032-L	3-Amino-1H-1,2,4-triazole	8	TTG162-L	Sesame seed oil unsaponified matter	71
TTG034-L	4-Ethoxynitrobenzene	12	TTG031-L	2-Chloro-4,6-dimethylaniline	7	TTG146-L	Forskolin	6	TTG044-L	Clofibrate	69
TTG166-L	Carbaryl	10	TTG044-L	Clofibrate	6	TTG062-L(C)	Dexamethasone	6	TTG074-L	Bromobenzene	47
TTG031-L	2-Chloro-4,6-dimethylaniline	10	TTG162-L	Sesame seed oil unsaponified matter	5	TTG054-L	Diethylnitrosamine (C57BL/6)	5	TTG109-L	Acephate	17
TTG141-L	Tributyltin x Clofibrate	9				TTG132-L	Curcumin	3	TTG160-L	5-fluorouracil	13
TTG032-L	3-Amino-1H-1,2,4-triazole	9				TTG136-L	Phytol	2			
TTG027-L	1,2,3-Triazole	9									
TTG160-L	5-fluorouracil	4									
	Sum Set (common)	75		Sum Set (common)	31		Sum Set (common)	46		Sum Set (common)	636
	Sum Set (Up)	59		Sum Set (Up)	22		Sum Set (Up)	23		Sum Set (Up)	636
	Sum Set (Dn)	16		Sum Set (Dn)	9		Sum Set (Dn)	23		Sum Set (Dn)	0
	PCP NOT Sum (unique to PCP)	23		PCP NOT Sum (unique to PCP)	24		PCP NOT Sum (unique to PCP)	81		PCP NOT Sum (unique to PCP)	556

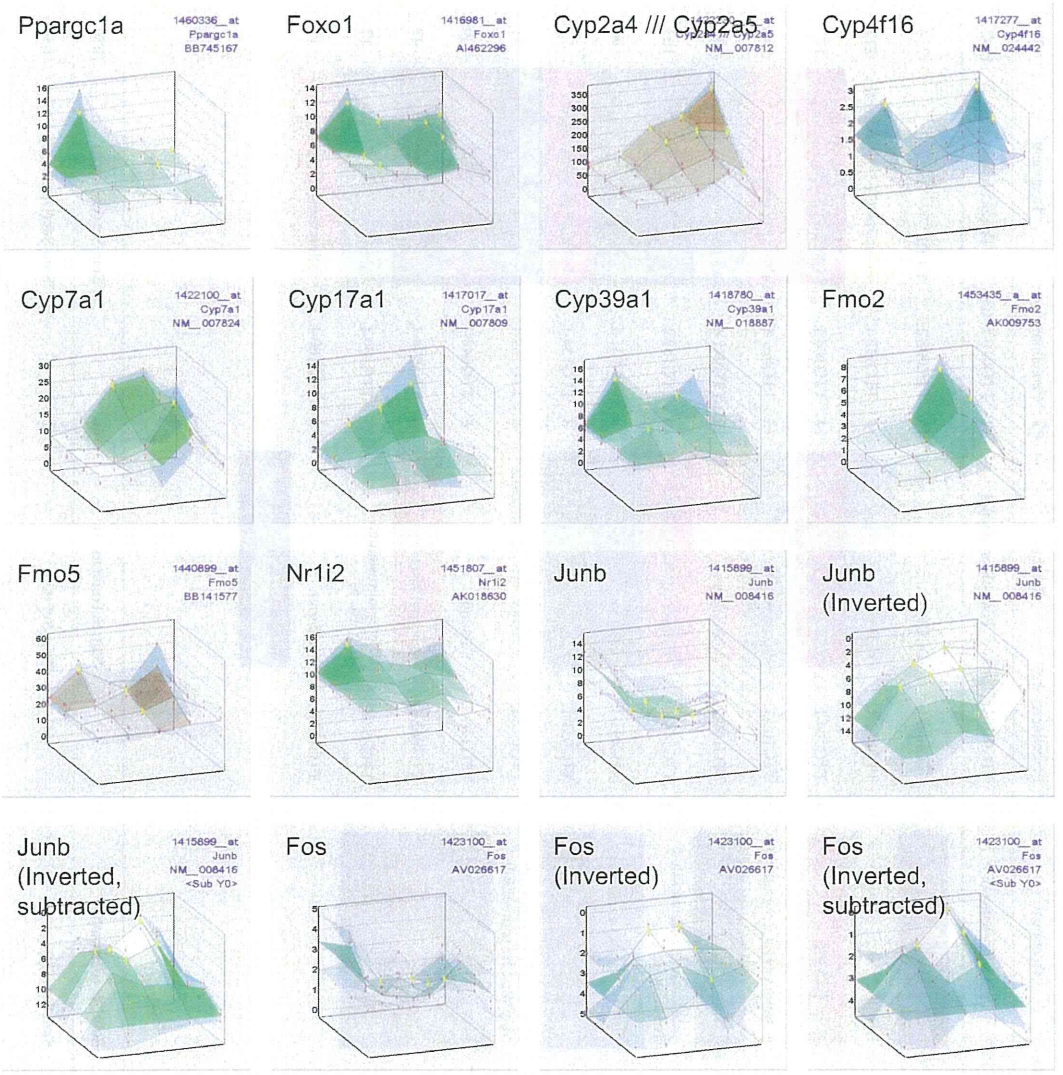

Fig. 3. Representative surface data of PSs induced at 2, 4 and 8 hr after PCP single gavage: Ppargc1a and Foxo1 are the members of gluconeogenesis pathway. A small set of genes of metabolic enzymes, such as Cyp2a4, Cyp4f16, Cyp7a1, Cyp17a1, Cyp39a1, Fmo2, and Fmo5 are induced during the first 8 hr. Nr1i2 or PXR/SXR is also induced. Down regulation of JunB and Fos at 2, 4, and 8 hr are noted. The graphs marked with (Inverted) are plotted with inverted z-axis, zero on top for better indication of suppression. The graphs with (Inverted, subtracted) are plotted, in addition to inverted z-axis, with the 2, 4, 8 and 24 hr values compensated by concurrent vehicle values so that the vehicle line is straight and cancels out the circadian changes.

other pattern recognition receptors (PRR), interferon regulatory factors (Irf) and interferon (Ifn) itself were included. These ISGs were uniquely induced by PCP. It is notable that inflammatory cytokines such as Tnf-α, IL-12 and CD40 were not effectively induced by PCP. The Ingenuity Pathways also plotted many genes in the interferon signaling with a very high probability score (Fig. 6).

In situ hybridization confirmed that hepatocytes were producing the Irf7 and Stat1 in a dose dependent manner (Fig. 7, only vehicle and top dose were shown).

The other half was assigned, by Ingeunity Pathway analysis, to Nrf2-mediated Oxidative Stress Responses

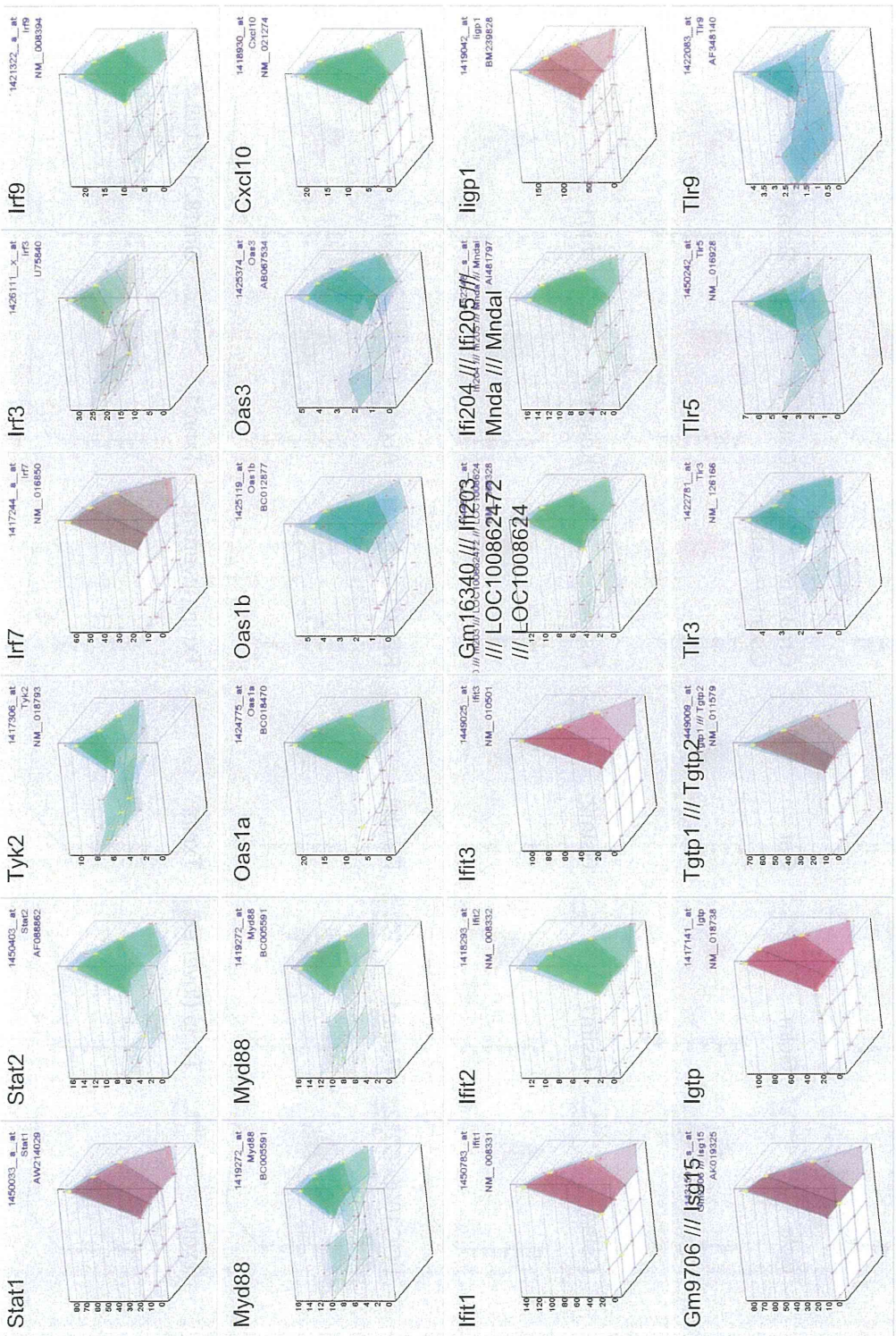

Fig. 4. Surface data of ISGs: Among about 1,200 PSs induced at 24 hr, half of them were uniquely induced by PCP and were assigned to ISG pathway from Stat1, Stat2, Tyk, to Irf7, Myd88, Oas, Ifit, Cxcl10 and other downstream targets. Some Tlrs were also uniquely up-regulated (cf. Supplementary Table 2).

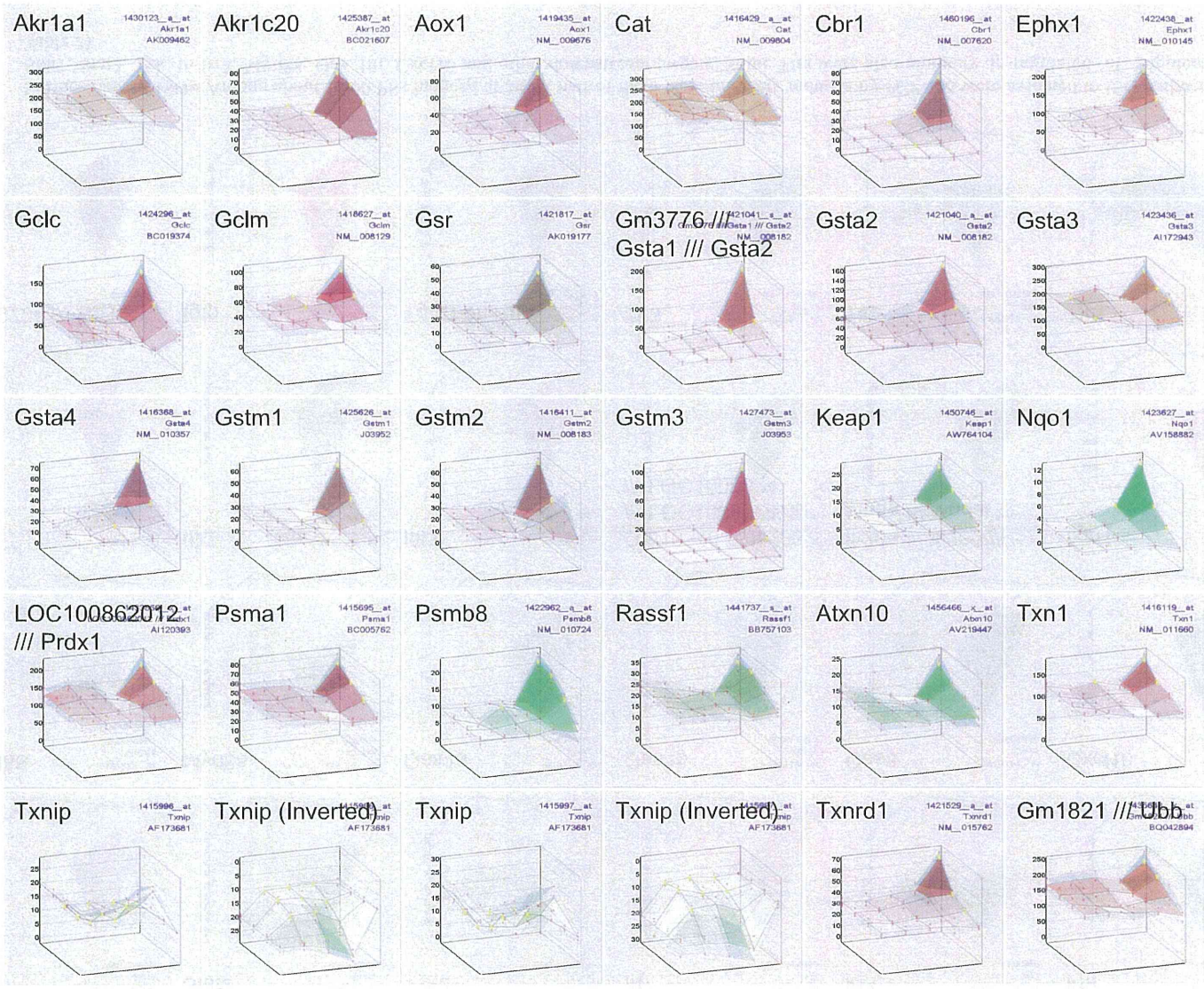

Fig. 5. Surface data of Nrf2-mediated oxidative stress response genes: Among about 1,200 PSs induced at 24 hr, another half of them were Nrf2-mediated oxidative stress response genes commonly induced by PCP and other 10 or so chemicals (cf. Supplementary Table 2). Nrf2 itself did not alter but Keap1 was clearly induced.

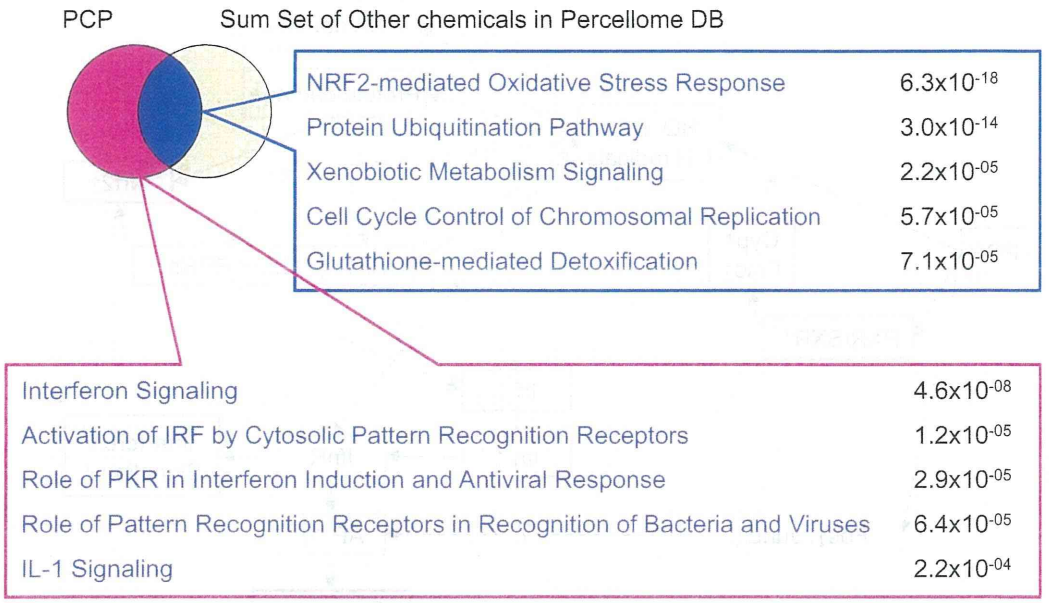

Fig. 6. Venn diagram of the PSs of PCP and sum set of other chemicals in Percellome Database. The PS list unique to PCP was assigned to Interferon signaling and related networks. The PSs induced by PCP and were shared by other chemicals in Percellome database were enriched in Nrf2-mediated oxidative stress response and Protein ubiquitination pathways. The names of the responses and their probability scores are generated by the Ingenuity Pathway analysis.

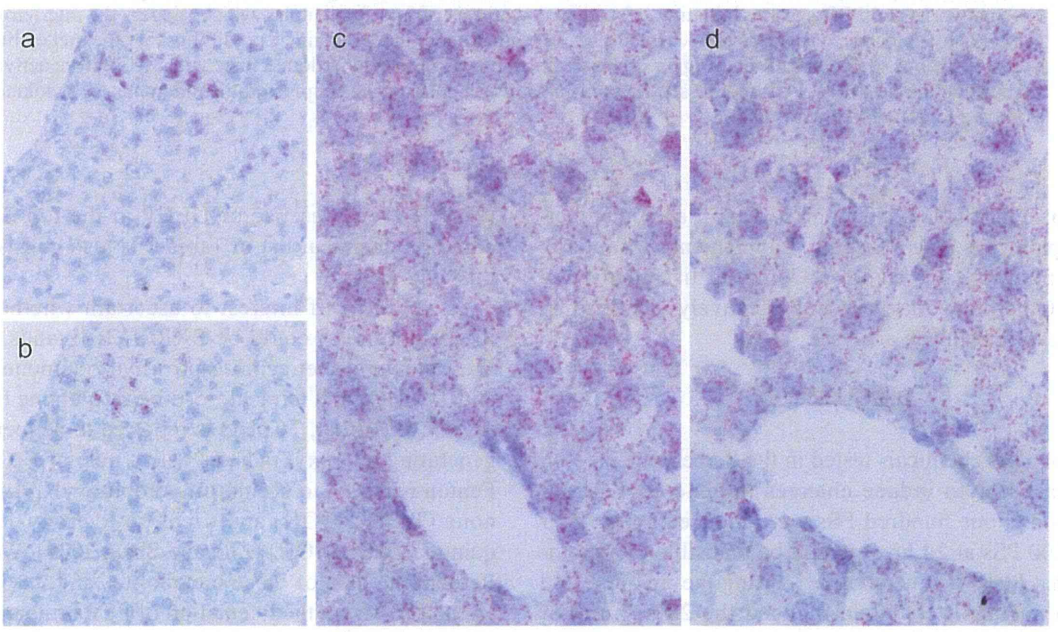

Fig. 7. *In situ* hybridization of Irf7 and Stat1. a) Vehicle control liver stained for Irf7. In a very low back ground, a small nest of hepatocytes was positively stained for Irf7. b) Vehicle control liver stained for Stat1. In a very low back ground, a small nest of hepatocytes was positively stained for Irf7. It is likely that the same hepatocyte is producing both mRNAs. c, d) High dose group stained for Irf7 and Stat1. Hepatocytes were shown to produce both mRNAs in a ubiquitous manner.