

Fine-tune a Large Language Model for food and feed safety

Lessons learned from the AI4NAMs project (OC/EFSA/SCER/2021/08)
“Exploring the use of Artificial Intelligence (AI) for extracting and integrating data obtained through New Approach Methodologies (NAMs) for chemical risk assessment”

01.12.2023, 90th EFSA Advisory Forum (Madrid)

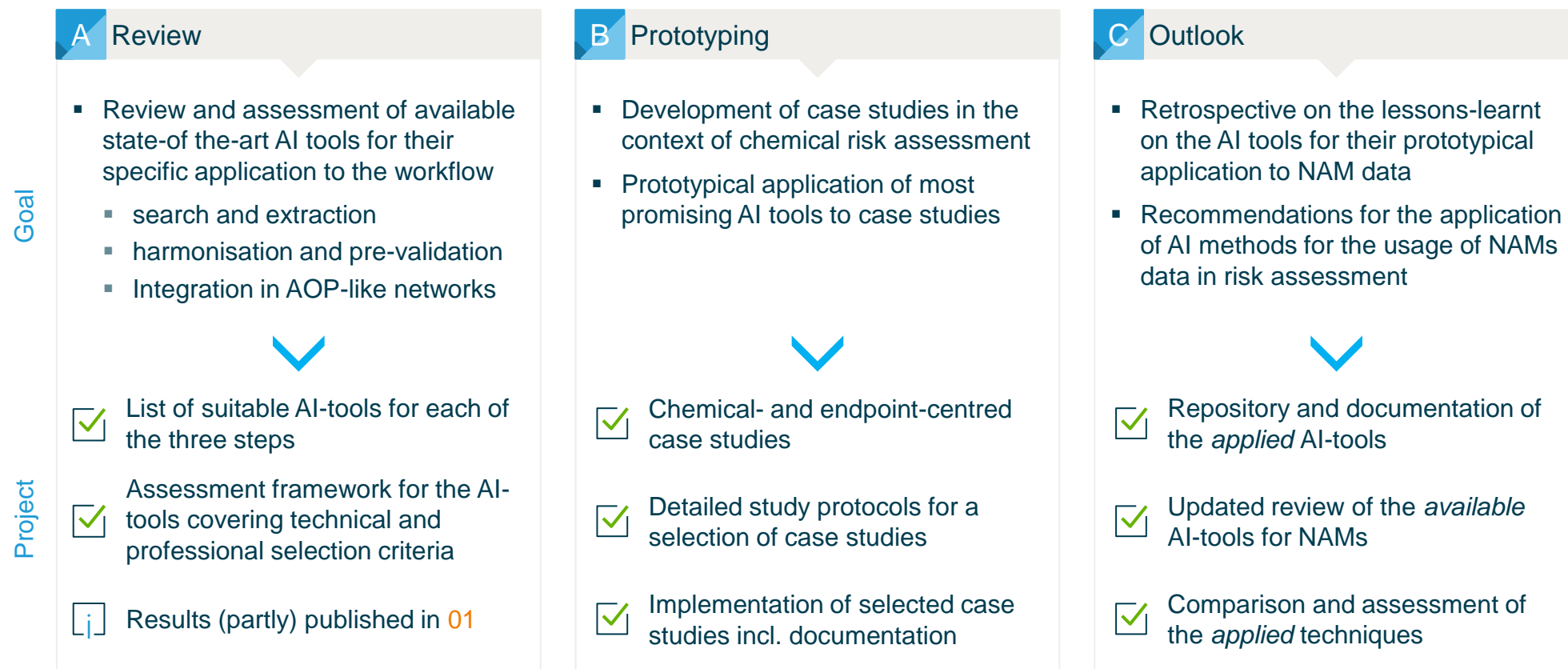
Carsten Kneuer

Toxicology of Active Substances
Department for Pesticide Safety (BfR)

Carla Mereu

Head of d-fine Italy

The project AI4NAMS, divided into three phases, has been completed in July 2023



Project consortium

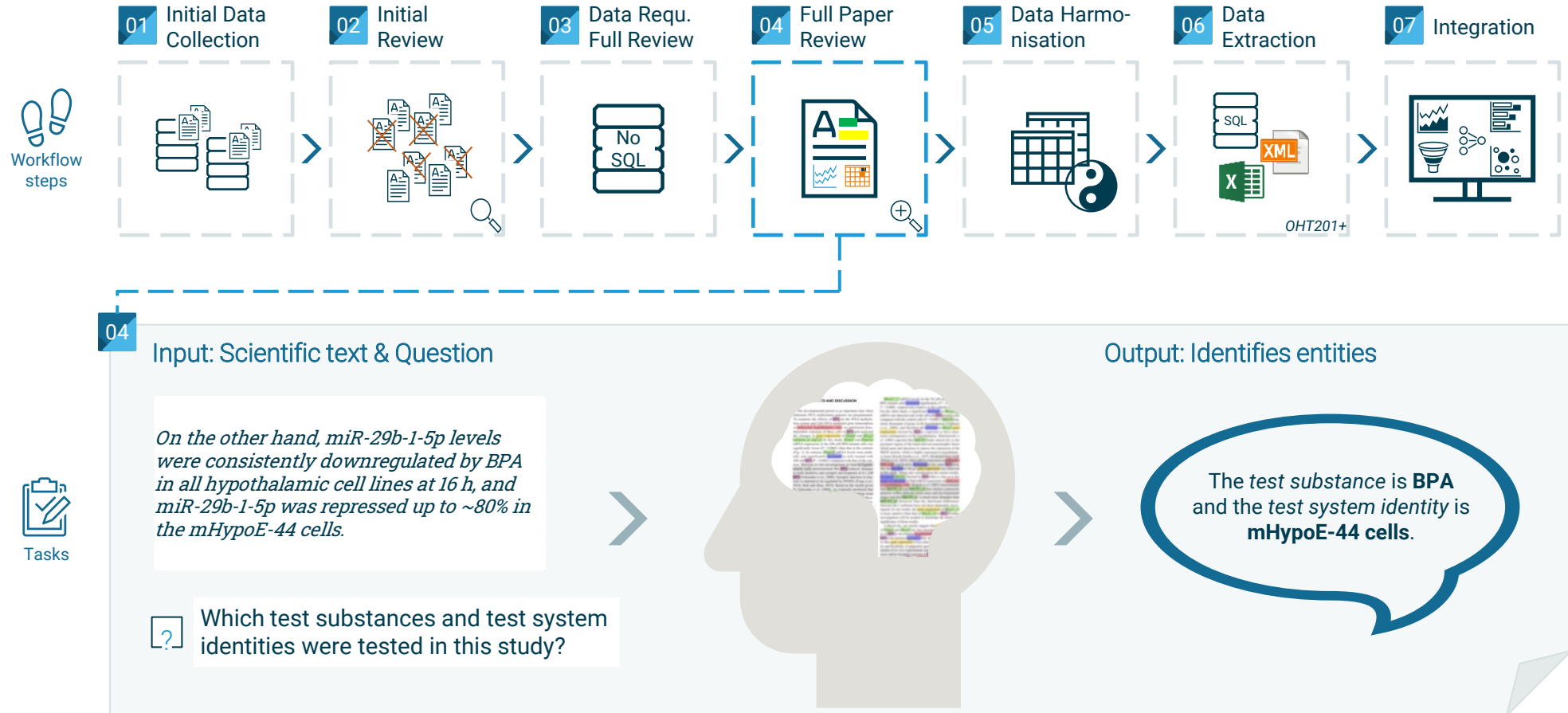
d-fine

 **BfR**
Bundesinstitut für Risikobewertung

 **WAGENINGEN**
UNIVERSITY & RESEARCH

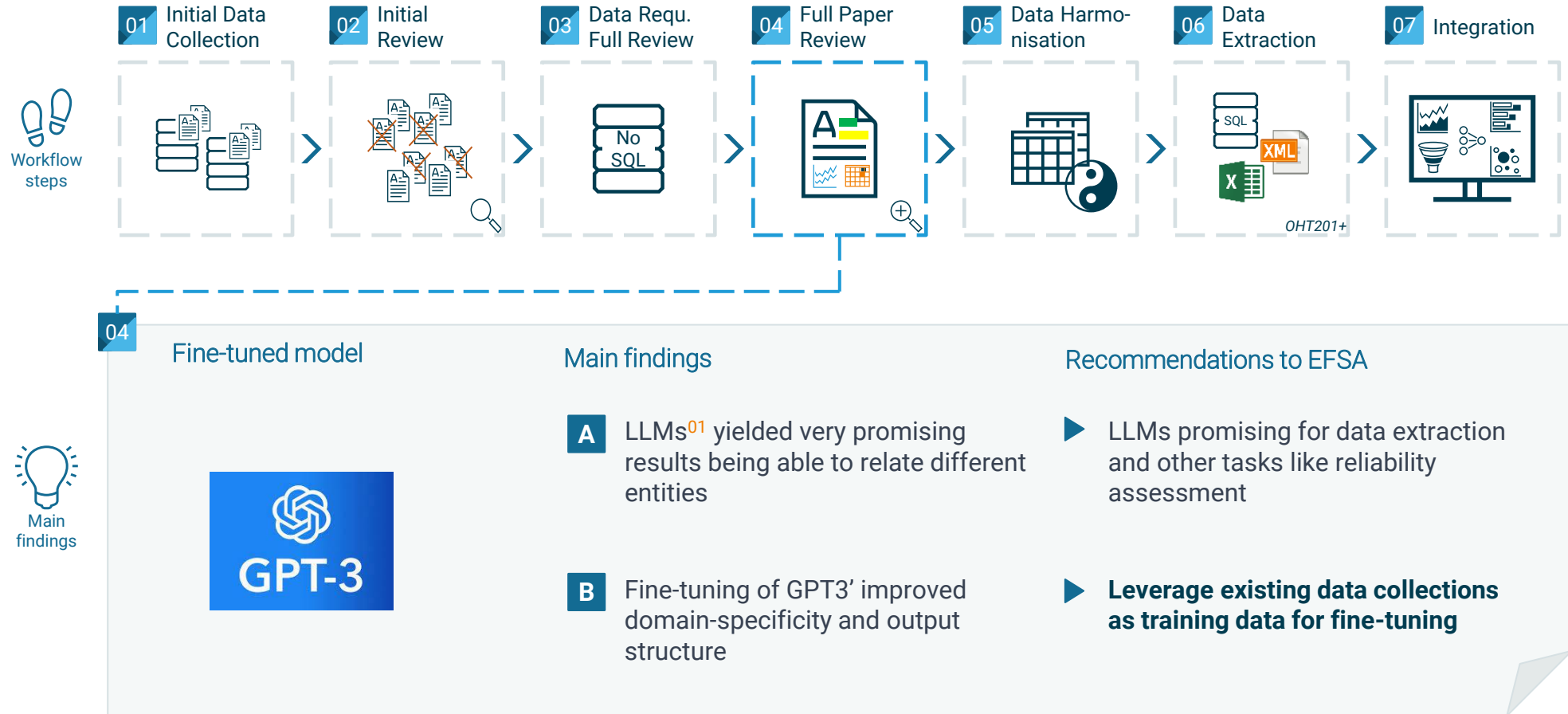
Goal of this project was to systematically explore the potential of applying AI-methods to NAM-data collection and integration, supporting better chemical risk assessment while reducing animal testing.

Selected AI⁰¹ tools and methods have been applied on NAM⁰²-data in a seven-step workflow, a crucial step was concerned with data extraction



Today we will focus on the result of the extraction exercise, focussing on the most promising approach resulted from the implementation of different combinations of tools within 6 case studies, i.e. the fine-tuning of LLMS⁰³.

Our project results highlighted the superiority of LLMs



⁰¹ Large Language Models

The accuracy and reliability of the models' results is not yet good enough to enable automation
But approaches can be found for the tools to best support the scientists' work.

Exemplary publication: Hoffmann et al. (2017)⁰¹

Focus: Results section



Test substance	Test system identity	Process	Object	Action
BPA	OVCAR-3 cells	cell proliferation		increase
BPA	KGN cells	cell proliferation		increase
BPA	OVCAR-3 cells	gene expression	GPR30	unaffected
BPA	KGN cells	gene expression	GPR30	unaffected
BPA,G15	OVCAR-3 cells	cell proliferation		reduced
BPA,G15	KGN cells	cell proliferation		reduced

⁰¹ <http://dx.doi.org/10.1016/j.tiv.2017.08.009>

Information retrieval

Asking for 2 entities

RESULTS AND DISCUSSION 01

The developmental period is an important time when elaborate DNA methylation patterns are programmed. To examine the effects of **BPA** on the DNA methylation system and CpG-DNA-mediated gene transcription in **embryonic hippocampal cells**, we performed dose-dependent exposures of these cells to **BPA** and analyzed the changes in **gene expression of *Dnmt1* and *Msp1***, **isoforms of *dnmt1* and *dnmt3***. In this study, ***Dnmt1* and *Dnmt3L* mRNA expression in the 200 μ M BPA-treated cells was significantly lower ($P < 0.0001$) than that in the controls (Fig. 1). In contrast, ***Dnmt3B* mRNA levels were markedly and significantly increased in cells treated with 200 μ M **BPA**** ($P < 0.0001$) compared with that of the controls. Previous *in vitro* investigations on fetal rat hippocampal cells demonstrated that **BPA** induces changes in both dendritic and synaptic development at 0.1 μ M **BPA** (Yokosuka *et al.*, 2008). Synaptic function in neurons is reported to be regulated by DNMTs (Feng *et al.*, 2010; Suen and Zhao, 2010). Based on the results given by Yokosuka *et al.* (2008), we originally predicted that **BPA** has some effect on ***Dnmt1*** at a concentration close to 0.1 μ M **BPA**. Contrary to our expectations, **BPA** affected the **gene expression of *Dnmt1*** at relatively high doses. Our results suggest that **BPA** has the potential to directly alter the **gene expression levels of *Dnmt1* in embryonic hippocampal cells**; however, these effects are not seen at the doses at which dendritic and synaptic development are affected. The previously reported effects of low doses of **BPA** on dendritic and synaptic development in **embryonic hippocampal cells** are considered to be induced by a mechanism other than alteration of **gene expression of *Dnmt1***. Interestingly, **gene expression of *Dnmt3a* and *Dnmt3b*** showed **opposite responses to **BPA****. Although both *dnmt3a* and *dnmt3b* are methyltransferases during the developmental period, **regulations of these genes by **BPA** may not contribute to one another**. Further research is needed to determine how **BPA** affects the responses, but it is suggested that alteration of **gene expression of *Dnmt1***, which are key players in the establishment of genomic methylation, may lead to the disruption of normal maturation of hippocampal function.**

WDR5 and *WDR5L* is located in the 2p and 12p arms of the human genome, respectively, and is expressed significantly ($P < 0.0001$ and $P < 0.0001$, respectively) relative to the controls (Fig. 2). On the other hand, a significant *WDR5* and *WDR5L* up-regulation was detected only in the 2p and 12p treated cells compared with the control cells ($P < 0.0001$ and $P < 0.0001$, respectively) (data not shown). In the transcriptional analysis of genes in the transcriptional analysis of human cells (Fig. 2), and therefore the *WDR5* and *WDR5L* genes up-regulation observed by *WDR5* is expected to have some consequences in the transcriptional mechanisms. Marikawa et al. (2001) reported that *WDR5* binds selectively to the promoter region of the brain-derived neurotrophic factor (*BDNF*) gene and functions to suppress the expression of the *BDNF* protein, which is highly expressed in hippocampus, a brain (Kawachi et al., 1997). Several papers by us (Shima et al., 2003) have shown expression of *WDR5* and *WDR5L* up-regulation in the same *WDR5* cells that the *WDR5* of *WDR5* gene up-regulation was observed in the cells. Taking into consideration the various results, *WDR5* and *WDR5L* observed by *WDR5* is also as in the results of *WDR5* and *WDR5L* expression in *WDR5* cells. *WDR5* and *WDR5L* (Shima et al., 2003) demonstrated the *WDR5* and *WDR5L* have distinct expression patterns within different brain areas and developmental stages, and that *WDR5* is much more abundant than *WDR5L*. However, the functional differences between the 2 isoforms have not been extensively investigated in our results. The *WDR5* and *WDR5L* is more sensitive than that of *WDR5* and *WDR5L* without investigation will be needed to determine the biological significance of these results.

Collectively, our results suggest that *WDR5* up-regulation of *WDR5* and *WDR5L* are less sensitive to lower doses of *WDR5* in developing *WDR5* cells. However, as *WDR5* is sensitive to *WDR5*, this again has the potential to alter *WDR5* and *WDR5L* has effects that generally inhibit and flexibility of synaptic gene expression. From the results of *WDR5* up-regulation being induced, such as *WDR5* and *WDR5L* up-regulation in *WDR5* is another effect consequences of *WDR5* in *WDR5* and *WDR5L* cells are directly expressed in function of various compound ligands, such as drug metabolism in the liver, that is, *WDR5* itself. However, alteration of *WDR5* and *WDR5L* in developing *WDR5* cells is expected as one of the sensitive factors of the genome is reported long-lasting adverse effects observed in the various treated with high doses of *WDR5* previously or now results.

Question

Which test substances and test system identities were tested in this study?

Answer - Experts

Test substance
BPA

Test system identity
embryonic hypothalamic cells

Plain model

Substance / Test System
BPA / Embryonic hypothalamic cells



Fine-tuned model

Test substance
BPA

Test system identity
mHypoEN44 cells⁰²



Information retrieval – example

Asking for 4 entities (1/2)

RESULTS AND DISCUSSION 01

The developmental period is an important time when elaborate DNA methylation patterns are programmed. To examine the effects of BPA on the DNA methylation system and CpG-DNA-mediated gene transcription in *embryonic hippocampal cells*, we performed dose-dependent exposure of these cells to BPA and analyzed the changes in *gene expression* of *Dnmt1* and *Mecp2* *Yokoyama et al. and al.* In this study, *Dnmt1* and *Dnmt3a* mRNA expression in the 200 μ M BPA-treated cells was significantly lower ($P < 0.0001$) than that in the controls (Fig. 1). In contrast, *Dnmt3b* mRNA levels were markedly and significantly *increased* in cells treated with 200 μ M BPA ($P < 0.0001$) compared with that of the controls. Previous *in vivo* investigations on fetal rat hippocampal cells demonstrated that BPA induces changes in both dendritic and synaptic development at 0.1 μ M BPA (Yokoyama et al., 2008). Synaptic function in neurons is reported to be regulated by DNMTs (Jiang et al., 2010; Sun and Zhao, 2010). Based on the results given by Yokoyama et al. (2008), we originally predicted that BPA has some effect on *Dnmt* at a concentration close to 0.1 μ M BPA. Contrary to our expectations, BPA affected the *gene expression* of *Dnmt* at relatively high doses. Our results suggest that BPA has the potential to directly alter the *gene expression* levels of *Dnmt* in *embryonic hippocampal cells*; however, these effects are not seen at the doses at which dendritic and synaptic development are affected. The previously reported effects of low doses of BPA on dendritic and synaptic development in *embryonic hippocampal cells* are considered to be induced by a mechanism other than alteration of *gene expression* of *Dnmt*. Interestingly, *gene expression* of *Dnmt1* and *Dnmt3a* showed *opposite responses* to BPA. Although both molecules act as de novo methyltransferases during the developmental period, impairment of these genes may not contribute to the similar. Further research is needed to determine how BPA affects the expression, but it is suggested that alteration of *gene expression* of *Dnmt*, which are key players in the establishment of genomic methylation, may lead to the disruption of normal maturation of hippocampal function.

Dnmt1 and *Dnmt3a* levels in the 20 μ M and 200 μ M BPA-treated cells were significantly ($P < 0.001$ and $P < 0.0001$, respectively) relative to the controls (Fig. 1). On the other hand, a significant increase in *Dnmt3b* mRNA was observed only in the 200 μ M-treated cells compared with the control cells ($P < 0.001$). BPA up-regulated thousands of genes in the hippocampus (Yokoyama et al., 2008), and therefore the *Dnmt3b* gene expression affected by BPA is expected to have some adverse consequences in the hippocampus. Nakamura et al. (2005) reported that *Dnmt3b* binds selectively to the promoter region of the brain-derived neurotrophic factor (BDNF) gene and functions to regulate the expression of the BDNF protein, which is highly expressed in hippocampus in mouse (Kawachi-Yamada et al., 2007). Some previous work (Watanabe et al., 2013) also reports expression of *Dnmt3b* in *embryonic hippocampal cells*. The up-regulation of *Dnmt3b* in the hippocampus may lead to the up-regulation of BDNF, which may contribute to the adverse results in this study. Using our model, we can predict the *gene expression* of *Dnmt1* and *Dnmt3a* is down-regulated by BPA, which is also up to the *gene expression* of *Dnmt3b* is up-regulated by BPA. Yokoyama et al. (2008) demonstrated that *Dnmt1* and *Dnmt3a* are more densely expressed in the hippocampus than *Dnmt3b*. Our model represents various entities within different brain areas and developmental stages and that *Dnmt1* and *Dnmt3a* are more densely expressed in the hippocampus than *Dnmt3b*. Therefore, these two functional differences between the 3 molecules have not been adequately represented. In our results, the *gene expression* of *Dnmt1* and *Dnmt3a* is down-regulated by BPA, which is also up to the *gene expression* of *Dnmt3b* is up-regulated by BPA. Further investigation will be needed to determine the biological significance of these results. Collectively, our results suggest that *gene expression* of *Dnmt1* and *Dnmt3a* is down-regulated by BPA, which is also up to the *gene expression* of *Dnmt3b* is up-regulated by BPA. This agrees with the potential to alter *gene expression* of *Dnmt* and may provide useful information for understanding of synaptic gene expression. Despite the results of *in vivo* experiments using rodents, such as to assess whether neuronal impairment is linked with demethylation of *Dnmt* and *Mecp2* in *embryonic hippocampal cells*, we directly reported the results of various compound log factors, such as drug metabolites in the data. Thus, the possible third adverse alteration of *gene expression* of *Dnmt1* and *Dnmt3a* is down-regulated by BPA, which is also up to the *gene expression* of *Dnmt3b* is up-regulated by BPA. Further investigation will be needed to determine the biological significance of these results.

Question

What are the test substances, biological processes, biological objects and corresponding actions identified in this study?

Answer - Experts

Test substance	Process	Object	Action
BPA	gene expression	Dnmt1	reduced
BPA	gene expression	Dnmt3a	decrease
BPA	gene expression	Dnmt3b	increased
BPA	gene expression	Mecp2_e1	increase
BPA	gene expression	Mecp2_e2	increase

Plain model

Test substance: BPA
Biological process: DNA methylation
Biological object: Dnmts and Mecp2
Action: BPA affects the gene expression of Dnmts and Mecp2



- * Model output partially correct, but
- Missing entries
 - No linkage between entities

Information retrieval – example

Asking for 4 entities (2/2)

RESULTS AND DISCUSSION 01

The developmental period is an important time when elaborate DNA methylation patterns are programmed. To examine the effects of **502** on the DNA methylation system and CpG-DNA-mediated gene transcription in **subependymal hyperplastic cells**, we performed dose-dependent exposure of these cells to **502** and analyzed the changes in **gene expression of Dnmt1 and Msp1** (Saito et al. and al.). In this study, **Dnmt1** and **Dnmt3a** mRNA expression in the 200 μ M **502**-treated cells was significantly lower ($P < 0.0001$) than that in the controls (Fig. 1). In contrast, **Dnmt3b** mRNA levels were markedly and significantly **increased** in cells treated with 200 μ M **502** ($P < 0.0001$) compared with that of the controls. Previous *in vitro* investigations on fetal rat hippocampal cells demonstrated that **502** induces changes in both dendritic and synaptic development at 5.1 μ M **502** (Yokosuka et al., 2008). Synaptic function in neurons is reported to be regulated by DNMTs (Feng et al., 2010; Saito and Zhao, 2010). Based on the results given by Yokosuka et al. (2008), we originally predicted the **502** has some effect on **Dnmt1** at a concentration close to 5.1 μ M **502**. Contrary to our expectations, **502** affected the **gene expression of Dnmt1** at relatively high doses. Our results suggest that **502** has the potential to directly alter the **gene expression levels of Dnmt1** in **subependymal hyperplastic cells**; however, these effects are not seen at the doses at which dendritic and synaptic development are affected. The previously reported effects of low doses of **502** on dendritic and synaptic development in **subependymal hyperplastic cells** are considered to be induced by a mechanism other than alteration of **gene expression of Dnmt1**. Interestingly, **gene expression of Dnmt3a** and **Dnmt3b** showed **opposite responses to 502**. Although both genes are up- or down-regulated during the developmental period, responsiveness of these genes to **502** may not coincide to one another. Further research is needed to determine how **502** affects the responses, but it is suggested that alteration of **gene expression of Dnmt1**, which are key players in the establishment of genomic methylation, may lead to the disruption of normal maturation of hippocampal function.

Shiga and *Shiga* exhibit toxicity in the 20 µM and 100 µM 95% ethanol cells (p -value: significantly ($p < 0.0001$) and $F = 0.0001$, respectively) relative to the control cells. On the other hand, a significant *Shiga* (p -value: *Shiga* 2.0) effect was observed only in the 200 µM 95% ethanol cells compared with the control cells ($p < 0.0001$, *Shiga* 2.0). Long-chain branched alcohols in the branched alcohols of *Shiga* *et al.* (2005), and therefore in *Shiga* (*Shiga* 2.0) *et al.* (2005) are reported to have strong consequences in the hepatocellular carcinogenesis. Maekawa *et al.* (2007) reported that *Shiga* 2.0 binds selectively to the promoter region of the toxin-derived microRNAs. Toxin-related gene and functions to improve the expression of the *Shiga* genes, which is highly expressed in hepatocellular carcinoma (Kawada *et al.* (2007), *Shiga* *et al.* (2007), *Shiga* *et al.* (2011)). *Shiga* effect expression in *Shiga* 2.0 (p -value: *Shiga* 2.0) in the same *Shiga* 2.0 that the *Shiga* of *Shiga* (*Shiga* 2.0) was observed in this study. Taking into consideration the current results, *Shiga* 2.0 (p -value: *Shiga* 2.0) effect is also as to the *Shiga* 2.0 (p -value: *Shiga* 2.0) effect expression in *Shiga* 2.0 (p -value: *Shiga* 2.0) (*Shiga* *et al.* (2007)). Moreover, the *Shiga* 2.0 (p -value: *Shiga* 2.0) and *Shiga* 2.0 (p -value: *Shiga* 2.0) have distinct expression patterns within different brain areas and developmental stages, and that *Shiga* 2.0 (p -value: *Shiga* 2.0) is much more abundant than *Shiga* 2.0 (p -value: *Shiga* 2.0). However, the functional differences between the 2 isoforms have not been thoroughly investigated. In our results, the *Shiga* 2.0 (p -value: *Shiga* 2.0) is more sensitive than that of *Shiga* 2.0 (p -value: *Shiga* 2.0). Further investigation will be needed to determine the biological significance of these results.

Collectively, our results suggest that *Shiga* 2.0 (p -value: *Shiga* 2.0) and *Shiga* 2.0 (p -value: *Shiga* 2.0) are less susceptible to toxin than *Shiga* 2.0 (p -value: *Shiga* 2.0). However, as *Shiga* 2.0 (p -value: *Shiga* 2.0) is, again, has the potential to alter *Shiga* 2.0 (p -value: *Shiga* 2.0) and play a role in growth and flexibility of synaptic gene expression. From the results of *in vivo* experiments using rodents, such as *in vivo* and/or neuronal expression in *Shiga* 2.0 (p -value: *Shiga* 2.0) is another effect concentration of *Shiga* 2.0 (p -value: *Shiga* 2.0). *Shiga* 2.0 (p -value: *Shiga* 2.0) is directly exposed to because of various compound drug factors, such as drug metabolism in the liver, blood, or genetic itself. However, alteration of *Shiga* 2.0 (p -value: *Shiga* 2.0) (*Shiga* *et al.* (2007)) in developing hepatocellular cells is reported as one of the causative factors of the peroxyl is reported long-lasting adverse effects observed in the rodents treated with high doses of *Shiga* 2.0 (p -value: *Shiga* 2.0) or *Shiga* 2.0 (p -value: *Shiga* 2.0).

Question

What are the test substances, biological processes, biological objects and corresponding actions identified in this study?

Answer - Experts

Test substance	Process	Object	Action
BPA	gene expression	Dnmt1	reduced
BPA	gene expression	Dnmt3a	decrease
BPA	gene expression	Dnmt3b	increased
BPA	gene expression	Mecp2_e1	increase
BPA	gene expression	Mecp2_e2	increase

Fine-tuned model

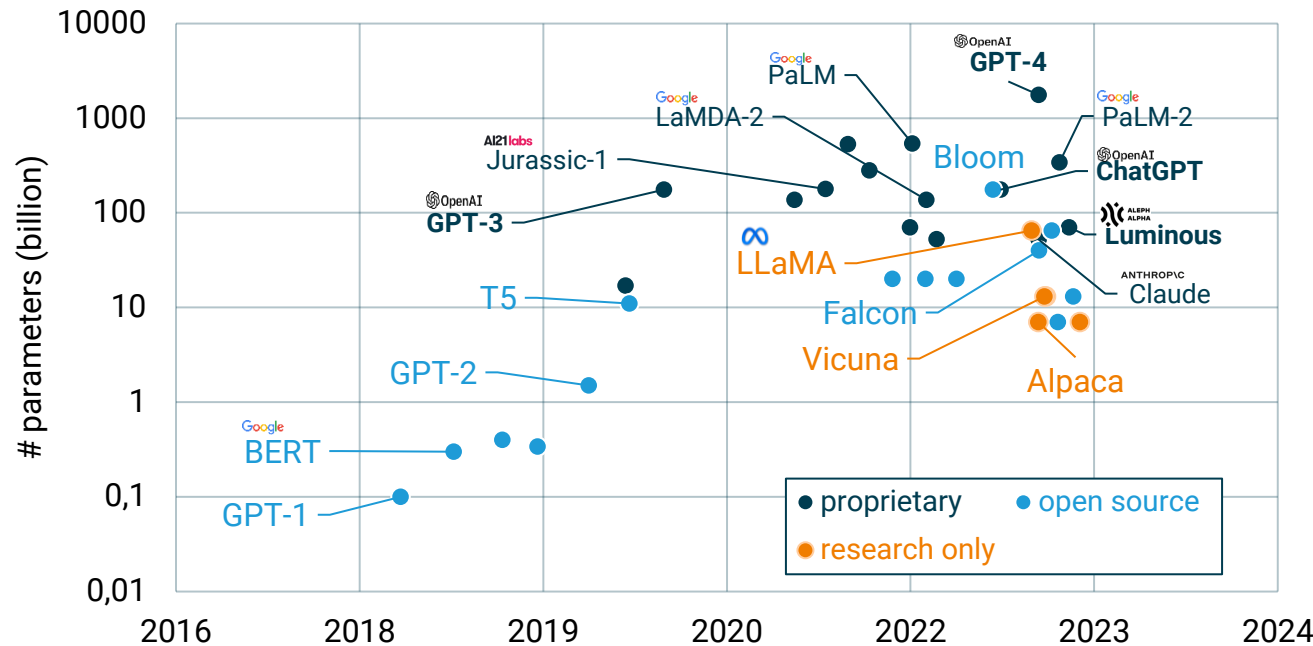
Test substance	Process	Object	Action
BPA	DNA methylation	nan	alteration
BPA	gene expression	Dnmt1	reduced
BPA	gene expression	Dnmt3a	increased
BPA	gene expression	Dnmt3b	increased
BPA	gene expression	Mecp2	increased
BPA	gene expression	Mecp2_e1	increase
BPA	gene expression	Mecp2_e2	increase
BPA	gene expression	Mecp2	decrease
BPA	gene expression	Mecp2_e1	decrease
BPA	gene expression	Mecp2_e2	decrease



The landscape of language models is very large and rapidly evolving

Selection of (large) language models

Some key players



- Dynamic field with many commercial and open-source initiatives
- Models have different scope, e.g. foundation models, instruction-tuned models and task-specific fine-tuned models
- Model size and training strongly impact performance
- Models differ significantly in terms of quality
- GPT-4 achieves the best overall quality at the moment

The optimal model selection depends on individual requirements such as task complexity, cost, speed, reliability, data privacy & security, customizability, ...

For an optimal domain-specific model, few-shot learning and fine-tuning can be used for optimizing the performance of LLMs

01 Question and prompt engineering

- Precise question that leave no / little room for interpretation
- Predefined answer ('Say "I don't know" if you do not know the answer.')
- Specify in which style the model should answer a question or specify the role of the model (e.g. "you are a customer service assistant")

capabilities  data  costs 

02 Prompt design

- Use separator sequences (such as '#####') to separate input from questions, examples and commands.
- Use meta-prompting, i.e. ask the LLM to improve the prompt

capabilities  data  costs 

03 Chain-of-thought (CoT)

- Instructions such as 'Let's think step by step' split the final answer into parts
- This increases the likelihood that the final answer will be consistent and correct
- Nested questions that build on previously given answers or use the previous answer as part of the question

capabilities  data  costs 

04 Few-shot learning

- Examples of question-answer pairs to specify the output
- Formatting of the answer (comma-separated, yes/no, ...) can be learned this way

capabilities  data  costs 

05 Retrieval augmented generation

- Combine LLMs with semantic search
- Search database for text and / or tabular data that is relevant for the query
- Enrich prompt with retrieved data

capabilities  data  costs 

06 Fine-Tuning

- Gradient-based fine tuning of model parameters
- Use of own (labelled) training data tailored to the application case

capabilities  data  costs 

Fine-tuning requires an investment in input and effort but can yield tangible advantages in selected cases



Definition – Fine-tuning⁰¹

Fine-tuning means supervised fine-tuning⁰², i.e. the process of *retraining pre-trained models on specific datasets*, typically to improve model performance on specific tasks or introduce information that wasn't well represented when the base model was originally trained.



Advantages of fine-tuning

- Higher quality results than what you can get just from prompt engineering.
- The ability to train on more examples than can fit into a model's max request context limit.
- Lower-latency requests, particularly when using smaller models.



Exemplary fine-tuning workflow⁰¹ (Azure / GPT)

- Prepare your training and validation data.
- Train your custom model.
 - Select a base model.
 - Choose your training data.
 - Optionally, choose your validation data.
 - Optionally, configure advanced options for your fine-tuning job.
 - Review your choices and train your new custom model.
- Check the status of your custom fine-tuned model.
- Deploy your custom model for use.
- Use your custom model.
- Optionally, analyze your custom model for performance and fit.

Exemplary training input

Text:

However, as BPA concentrates in the placenta, the fetus is exposed to a high concentration of BPA. BPA has the potential to act as an endocrine disruptor and interfere with the normal function of key players that provide steady-state epigenetic gene regulation.

Correct answer:

Test substance
BPA

Text:

In our results, the gene expression levels of Dnmts in embryonic hypothalamic cells; however, these effects are not seen at the doses at which dendritic and synaptic development are affected.

Correct answer:

Test substance
BPA

Text:

Our results suggest that BPA has the potential to directly alter the gene expression levels of Dnmts in embryonic hypothalamic cells; however, these effects are not seen at the doses at which dendritic and synaptic development are affected.

Correct answer:

Test substance
BPA

⁰¹ cf. <https://learn.microsoft.com/en-us/azure/ai-services/openai/concepts/fine-tuning-considerations>

⁰² To be distinguished from continuous pre-training or Reinforcement Learning through Human Feedback (RLHF)



Contacts



Dr Carsten Kneuer

Head of Unit, Toxicology of Active
Substances and Their Metabolites, BfR
Tel +49 30 1841226300
Carsten.Kneuer@bfr.bund.de



Dr Carla Mereu

Head of d-fine Italy
Tel +39 348 1988969
Mobile +39 348 1988 969
Carla.Mereu@d-fine.com
