# Statistical Approaches for Assessing Health Effects of Environmental Mixtures in Epidemiological Studies

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I have posted all material (slides, R code, and 2 datasets) on abellavia.altervista.org (under "Research")

The R file includes code to replicate all presented results, based on analyses of dataset 1. Dataset 2 is for you in case you want to practice on another example.

# Motivating example - mixtures of phthalates and birthweight

Contents lists available at ScienceDirect

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## Evaluating effects of prenatal exposure to phthalate mixtures on birth weight: A comparison of three statistical approaches

Check for updates

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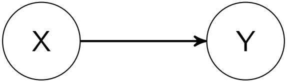
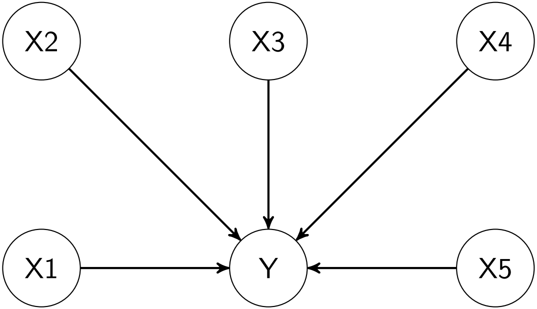
- Recent findings suggest that environmental exposures may contribute in increasing the risk of several pregnancy complications
- Phthalates, for instance, are ubiquitous (e.g. in food, plasticizers, cosmetics, personal care products, toys) chemical disruptors that are able to interfere with the endocrine system and other biological mechanisms
- There are around 10-15 phthalate metabolites commonly used in industry, especially as plasticizers or fragrance-aids. Negative effects on health have been demonstrated for a large number of these individual metabolite
- Humans, however, are simultaneously exposed to a set of chemicals, rather than one-at-the-time. Such chemicals can present interactions, and need to be evaluated as a simultaneous exposure (that is, as a chemical mixture)

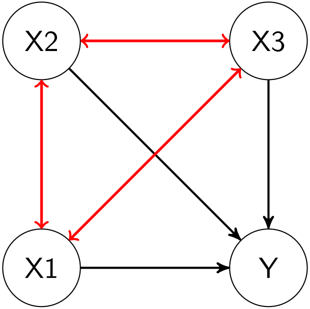
In practical terms, this is the approach commonly used in epidemiology and clinical research. We evaluate the effect (or association) between one risk factor and the health outcome of interest.



This, however, is the real-world scenario we generally have when investigating environmental exposures (for example, several phthalate metabolites simultaneously and possibly synergistically contributing to the development of the disease)



Environmental exposures, in general, are highly correlated, present potential interactions, and act as a confounder of each-other association.



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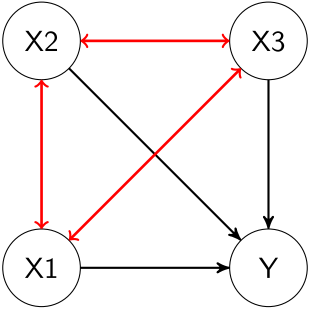


As such, the optimal statistical approach to evaluate the effect of these covariates on $Y$ must simultaneously include them in the same statistical model

Standard approach: mutually adjust for all predictors in the same statistical model

$$f(Y) = \beta_0 + \beta_1 \cdot X_1 + \beta_2 \cdot X_2 + \beta_3 \cdot X_3$$

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This approach, however, is subject to two important limitations:

- Collinearity. This arises when exposures are highly correlated within each other
- Overfitting. As the number of exposures (and potential interactions) increases this becomes a crucial issue

# Few more words on collinearity

- Including multiple correlated covariates in the same statistical model can lead to the statistical issue of multicollinearity (or simply collinearity).
- If the correlation between two covariates (say $X_1$ and $X_2$) is very high, then one is a pretty accurate linear predictor of the other

# Few more words on collinearity

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- If the correlation between two covariates (say $X_1$ and $X_2$) is very high, then one is a pretty accurate linear predictor of the other
- The associated coefficients may be highly unreliable
- Of important note, collinearity does not influence the overall performance of the model, but has an important impact on individual predictors. Unfortunately, this is exactly what we are interested in

Note that these two problema are common in environmental epidemiology, but can also arise in several other settings, for example:

- Nutrients
- Dietary factors
- Psychology
- Anthropometric factors (e.g. height and BMI)

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# Classification of statistical approaches for environmental mixtures

Several (at least 25-30) approaches have been proposed in the last decades (listed in Taylor et al., 2016). They can be grouped in 4 major categories:

- Classification and prediction
- Variable selection
- Variable shrinkage
- Exposure-response surface estimation

| | |
|---|---|
| Single chemical analysis | Classic linear regression (ordinary least squares) |
| Multiple regression | Classic linear regression (ordinary least squares) |
| Visualization, structural equation modeling (SEM), and principal component analysis (PCA) | Classification and prediction |
| Informed sparse PCA and segmented regression | Classification and prediction |
| Bayesian g-formula | Classification and prediction |
| PCA | Classification and prediction |
| Classification and regression trees (CART) | Classification and prediction |
| Bayesian profile regression | Classification and prediction |
| Random forest | Classification and prediction |
| Multivariate adaptive regression splines (MARS) | Classification and prediction |
| Bayesian non-parametric regression | Classification and prediction |
| Bayesian additive regression trees (BART) and negative sparse PCA (NSPCA) | Classification and prediction |
| Conformal predictions | Classification and prediction |
| Bayesian kernel machine regression (BKMR) | Exposure–response surface estimation |
| Building Bayesian networks | Exposure–response surface estimation |
| Exposure surface smoothing (ESS) | Exposure–response surface estimation |
| Modes of action (results presented for $Z=0$ strata) | Other |
| Feasible solution algorithm (FSA) | Other |
| Exploratory data analysis (EDA) | Other |
| Novel approach and least-angle regression (LARS) | Variable selection |
| Machine learning | Variable selection |
| Two-step variable selection and least absolute shrinkage and selection operator (LASSO) | Variable selection |
| Two-step shrinkage-based regression | Variable selection |
| Factor mixture models | Variable selection |
| Subset and bootstrap | Variable selection |
| Variable selection regression (VSR) | Variable selection |
| Bayesian estimation of weighted sum | Variable shrinkage strategies |
| Shrinkage methods (LASSO/LARS) | Variable shrinkage strategies |
| Weighted quantile sum regression (WQS) | Variable shrinkage strategies |
| LASSO | Variable shrinkage strategies |

# Illustrative examples

Two simulated datasets (dataset1.xls, dataset2.xls) are provided in the workshop material. All results presented are based on Dataset2 (R code also provided). You can use the other for individual practice
In this workshop we will focus on:

- Regression-based approaches and their limitations
- Classification approaches
- Introducing BKMR, a recently developed and (in my opinion) very powerful method for environmental mixtures

# 1. Standard approach

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$$Y = \beta_0 + \beta_1 \cdot X + \sum_{i=1}^{3} \beta_{i+1} \cdot Z_i$$

## Mutually adjusted regression

$$Y = \beta_0 + \sum_{i=1}^{14} \beta_i \cdot X_i + \sum_{i=1}^{3} \beta_{i+14} \cdot Z_i$$

Multiple testing corrections (e.g. Bonferroni, FDR) should be considered when the latter is used

# Multiple regression - Results

| | $\beta$ (one at the time) | p-value | $\beta$ (mutually adjusted) | p-value |
|---|---|---|---|---|
| $X_1$ | 0.11 | $< 0.001$ | 0.06 | 0.08 |
| $X_2$ | 0.07 | 0.01 | 0.02 | 0.55 |
| $X_3$ | 0.08 | 0.007 | -0.03 | 0.77 |
| $X_4$ | 0.09 | 0.003 | 0.05 | 0.64 |
| $X_5$ | 0.07 | 0.005 | 0 | 0.92 |
| $X_6$ | 0.12 | $<0.001$ | 0.06 | 0.47 |
| $X_7$ | 0.15 | $<0.001$ | -0.03 | 0.62 |
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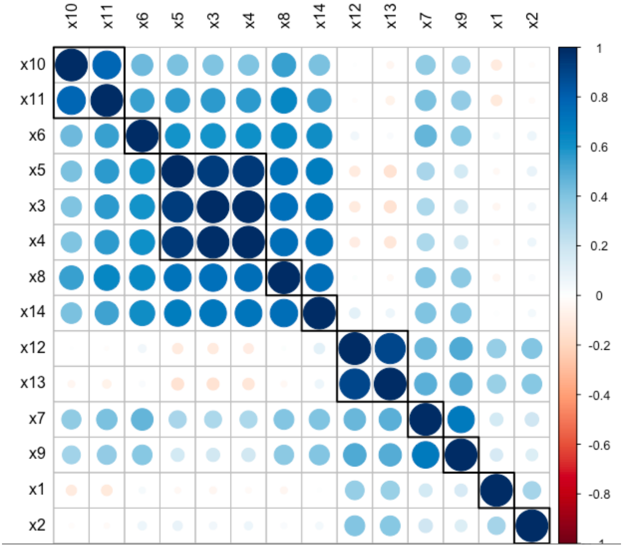
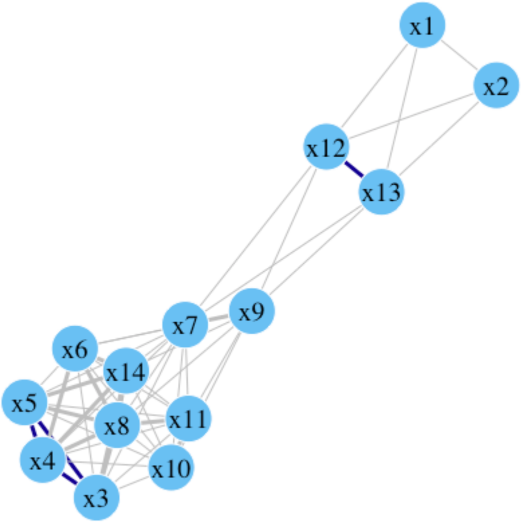
# What about the correlation?



There are multiple ways for investigating the correlation structure. Networks are another useful tool



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- Classification approaches reduce collinearity by identifying *orthogonal factors* that summarize a set of covariates of interest

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- A common practical advice is to select a number of components that jointly explain around 80% of the original variance
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- Accessible and brief introduction: `http://www.lauradhamilton.com/introduction-to-principal-component-analysis-pca`

# PCA - example

- Can be run with *principal* command within the *psych* library in R
- We run multiple PCA models by changing the number of components, and then focus on the explained variance to select the best one
- It is always recommended to rescale the covariates (mean 0, unit sd) before running a PCA model

# PCA - 3 components

Explained variance=74% (39 %, 19%, 16%, respectively)

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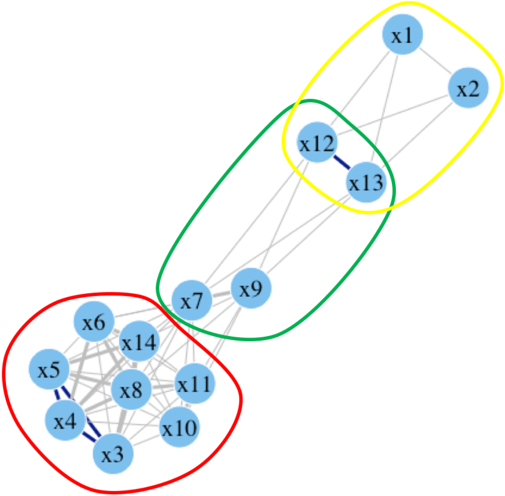
| Loading factors | | | |
|---|---|---|---|
| | 1st | 2nd | 3rd |
| $X_1$ | 0.00 | 0.05 | **0.66** |
| $X_2$ | 0.12 | -0.01 | **0.71** |
| $X_3$ | **0.96** | -0.01 | 0.01 |
| $X_4$ | **0.97** | 0.00 | 0.02 |
| ... | ... | ... | ... |
| $X_{12}$ | -0.16 | **0.56** | **0.70** |
| ... | ... | ... | ... |

# PCA - 3 components

1st - red; 2nd - green; 3rd - yellow



# PCA - 4 components

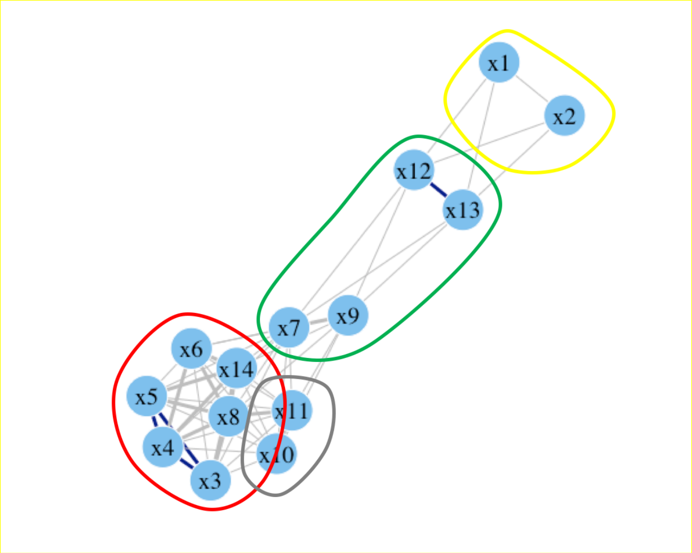
Explained variance=80% (35 %, 20%, 14%, 11%, respectively)

Loading factors

| | 1st | 2nd | 3rd | 4th |
|---|---|---|---|---|
| $X_1$ | -0.05 | 0.19 | -0.01 | **0.74** |
| $X_2$ | 0.07 | 0.14 | -0.03 | **0.81** |
| $X_3$ | **0.96** | -0.05 | 0.14 | 0.02 |
| $X_4$ | **0.96** | -0.04 | 0.15 | 0.03 |
| ... | ... | ... | ... | |
| $X_{11}$ | **0.48** | 0.08 | **0.79** | -0.05 |
| $X_{12}$ | -0.09 | **0.82** | -0.12 | **0.39** |
| ... | ... | ... | ... | |

# PCA - 4 components

1st - red; 2nd - green; 3rd - yellow, 4th - grey



# PCA - Regression

3 components

$$Y = \beta_0 + \beta_1 \cdot PC_1 + \beta_2 \cdot PC_2 + \beta_3 \cdot PC_3 + \sum_{i=1}^{3} \beta_{i+3} \cdot Z_i$$

| | $\beta$ | p-value |
|---|---|---|
| $PC_1$ | 0.18 | $< 0.001$ |
| $PC_2$ | 0.11 | $< 0.001$ |
| $PC_3$ | 0.04 | 0.06 |

4 components

$$Y = \beta_0 + \beta_1 \cdot PC_1 + \beta_2 \cdot PC_2 + \beta_3 \cdot PC_3 + \beta_4 \cdot PC_4 + \sum_{i=1}^{3} \beta_{i+4} \cdot Z_i$$

| | $\beta$ | p-value |
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| $PC_1$ | 0.16 | $< 0.001$ |
| $PC_2$ | 0.08 | $< 0.001$ |
| $PC_3$ | 0.05 | 0.01 |
| $PC_4$ | 0.10 | $< 0.001$ |

# b) Structural Equation Model

- PCA could be integrated within a SEM framework.
- The main contribution of SEM in a PCA environment is a better account for measurement error

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- PCA could be integrated within a SEM framework.
- The main contribution of SEM in a PCA environment is a better account for measurement error
- In this case (you can check the code and results), we get exactly the same coefficients estimated with PCA

# Summary of classification approaches

- + They **reduce data dimension**
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- + They **reduce data dimension**
- + A certain amount of flexibility is allowed
- - Subjective choice of components
- - Difficult interpretation if groups with a specific biological meaning are not identified
- - Difficult to retrieve the individual contribution of the predictors
- - Difficult, sometimes impossible, to account for non-linear effects

# 3. Exposure surface estimation - Bayesian Kernel Machine Regression

- BKMR (Bobb et al., 2015) was (mainly) motivated by the last two final points (i.e. predictor-specific contribution, non-linear effects)

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- BKMR (Bobb et al., 2015) was (mainly) motivated by the last two final points (i.e. predictor-specific contribution, non-linear effects)
- Kernel machine regression are a popular tool in machine learning
- The main idea is that we model the relationship between a high-dimensional set of $M$ predictors and the outcome by using a flexible function $h$ of the covariates

$$g(E(Y_i)) = h(x_{i1}, ..., x_{iM}) + \beta \cdot Z_i$$

- *h* is called *exposure-response* function, and is estimated with a Gaussian Kernel, which flexibly captures a wide range of underlying functional forms for *h*
- BKMR implements Markov Chain Monte Carlo (MCMC, an iterative estimation algorithm) for the estimation of the function (n.b. it takes long)

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- BKMR implements Markov Chain Monte Carlo (MCMC, an iterative estimation algorithm) for the estimation of the function (n.b. it takes long)
- One can also provide preliminary information to the model (such as the grouping of the predictors that we have already seen).
- In the following slide we will however proceed in a non-informative environment.

# BKMR - predictor-response function

- Estimation is straightforward. The package *bkmr* does everything, just write one line and wait few minutes
- Different tools are then available to summarize results
- Introduction to the package: `https://jenfb.github.io/bkmr/overview.html`

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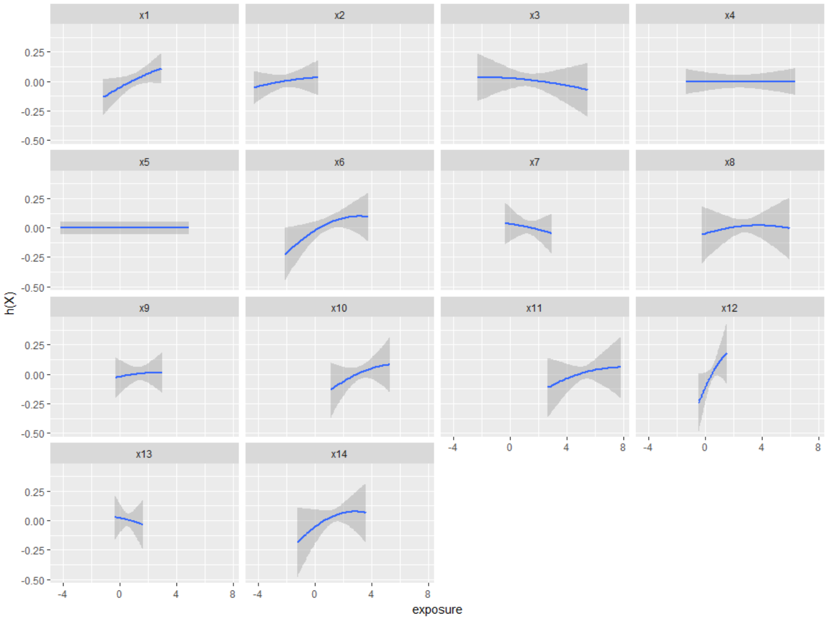
- Different tools are then available to summarize results
- Introduction to the package: `https://jenfb.github.io/bkmr/overview.html`
- Clearly, it is not possible to visualize the entire function $h$ (in our example, this is a 14-dimension function)
- The best way of presenting results is to visualize the relationship between 1 predictor and the outcome while fixing the other to a specific value (e.g. the median)


x1
x2
x3
x4
x5
x6
x7
x8
x9
x10
x11
x12
x13
x14
h(X)
0.25
0.00
-0.25
-0.50
-4
0
4
8
exposure

# (These were the original results)

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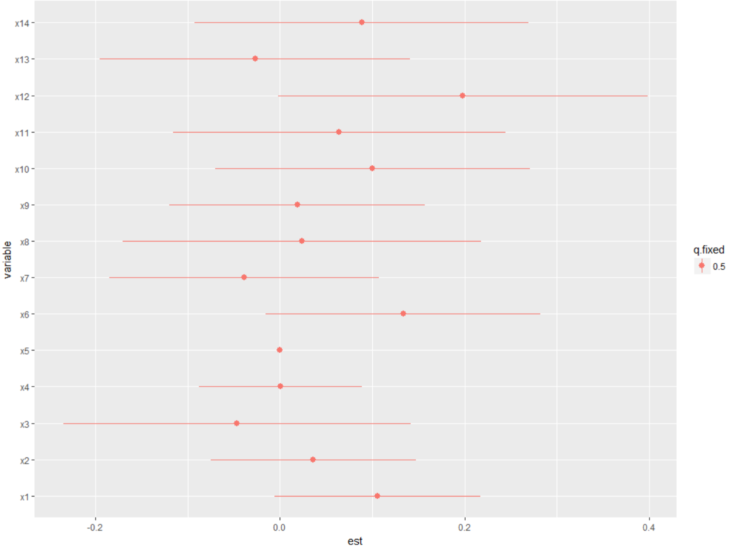
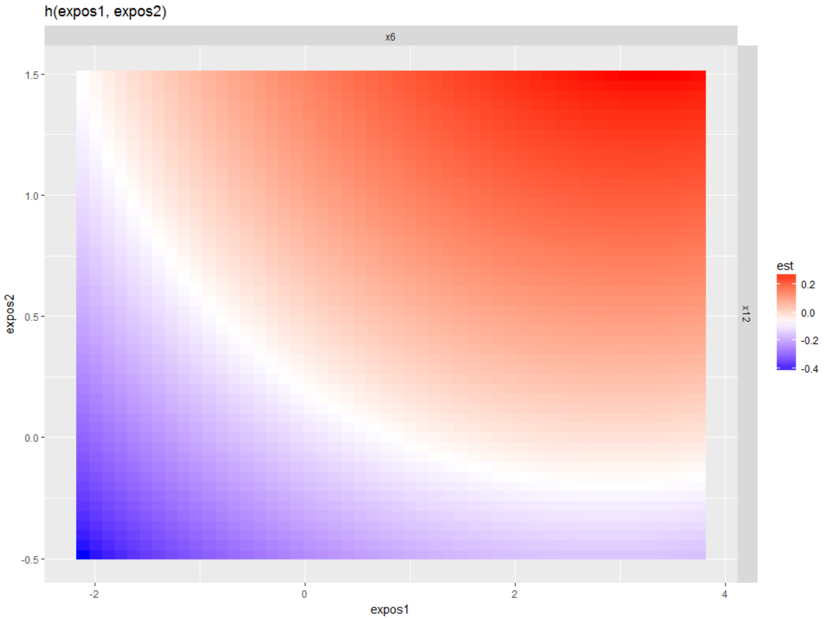
We could also plot the effects for a specific change in the predictors (e.g. from the 10th to the 90th percentile). This is the best summary plot if all relationships are linear



# BKMR - bivariate predictor-response function (interactions)

- We may be interested in the joint effect of two predictors, while fixing the other to a predefined value (eg. the median)
- We could focus, for example, on those predictors where we observed the strongest individual effects ($X_6$ and $X_{12}$)


h(expos1, expos2)
x6
x12
expos2
expos1
1.5
1.0
0.5
0.0
-0.5
-2
0
2
4
est
0.2
0.0
-0.2
-0.4

# Summary

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- In real-world situations humans are generally exposed to a mixture of risk factors. This is most common in evaluating environmental exposures.
- Evaluating mixtures with standard regression approaches may be problematic due to issues such as collinearity and overfitting
- In our example, standard regression models showed a lot of significant results, while through mixture methods we found that only 2/3 exposures had a significant effect. The other individual effects were a results of the high correlation with these 2/3 main contributors

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- Evaluating mixtures with standard regression approaches may be problematic due to issues such as collinearity and overfitting
- In our example, standard regression models showed a lot of significant results, while through mixture methods we found that only 2/3 exposures had a significant effect. The other individual effects were a results of the high correlation with these 2/3 main contributors
- Several methods have been presented, all of them with strengths and limitations (that is, we don't have an optimal method)
- Classification approaches (e.g. PCA) reduce dimensionality and are useful if biological groups (i.e. exposures that are expected to have the same behavior with respect to the outcome) are clearly identified
- However, they fail to identify the specific contribution of each predictor and cannot take into account non-linear effects

# Summary (2)

- BKMR, while taking into account the grouping of the exposure, addresses these two limitations, thus proposing itself as a complete and flexible method
- Several alternative methods are available. These were not covered only because of time and may provide additional advantages in specific settings.

# Are all studies I published so far wrong?

# Are all studies I published so far wrong?

- Definitely not, but they may not have taken into account the full picture
- Use your previous findings as a starting point for your first mixture model

# Main References


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