Assessing the Risk of Non-Threshold Carcinogens

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The European Food Safety Authority (EFSA) adopted an opinion on substances that are both genotoxic and carcinogenic" proposing use the MOE for risk assessment.

SCHER/SCCP/SCENIHR have been asked:

1) To critically review the available methodologies and approaches for the risk assessment of such substances

2) If possible, to identify a harmonised methodology/ approach for their risk assessment

3) To critically review the available methodologies and approaches to identify genotoxic carcinogens in the absence of long term carcinogenicity studies

4) If possible, to identify a harmonised methodology/ approach for their risk assessment

5) Thresholds for genotoxic carcinogens?

Approaches for risk assessment of genotoxic carcinogens

1. Linear Extrapolation from High Doses in Repeated Dose Animal Studies to Low Dose Human Exposure

2. Margin of Exposure (MOE)

3. ALARA

4. Threshold of Toxicological Concern (TTC)

5. (Q)SAR for the assessment of mutagens and carcinogens

6. Use of epidemiological studies in cancer quantitative risk assessment

1. Linear extrapolation from high to low doses

Three different methods used in Europe and USA.

The "Linearised Multistage Model" (US EPA)

Later: "LED10 method" (EPA), "T25 method" (Europe)

The two latter consider linear dose response between tumour formation and exposure and involve linear extrapolation from a dose descriptor (BMDL or T25) to human exposure.

The results obtained with the two latter extrapolation methods are similar, mostly.

2. Margin of Exposure (MOE)

MOE: Difference between dose descriptor for tumour formation in animals (or humans) and human exposure.

- Requires reliable animal (or epidemiology) carcinogenicity data and good quality exposure assessment.

- Depending on the quality of the animal carcinogenicity data and the number of dose levels, the T25 or the BMD(L) are used as dose descriptors.

- MOE of 10,000 or more, based on the BMDL10 are "of low concern from a public health point of view and might be reasonable considered as a low priority for risk management action".

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3. As Low As Reasonably Achievable (ALARA)

The ALARA principle keeps the exposure to carcinogenic substances at the lowest achievable level, usually limited by technological limitations or economic considerations.

Advantage:

Only hazard identification data needed and exposure to genotoxic and carcinogenic substances is limited to technically unavoidable amounts.

Disadvantage:

No use of the general toxicological database, not useful for risk comparison, no consideration of carcinogenic potency and the actual exposures (which may result in very low risks).

The principle deals with compounds of unknown toxicity. Based on chemical structure, human exposure threshold values are established.

Genotoxic Carcinogens: 0.15 µg/day

Organophosphates: 18 µg/day

All other substances: 90, 540 or 1,800 µg/day for Cramer classes I, II and III (Munro et al 1996, Kroes et al. 2004).

The recently proposed TTC concept for cosmetics considers rates of dermal absorption and duration of exposure (Kroes et al 2007).

Numerous attempts have been made to create alternative predictive in silico models.

Given the structural diversity and possible complexity of chemicals and the huge range and variability of possible interactions of chemicals in biological systems, it is highly unlikely that (Q)SAR models will achieve absolute certainty in predicting carcinogenicity.

(Q)SAR software programs provide the possibility of cost effective screening for possible carcinogenic effects.

Dose-response information from epidemiological studies are preferred for quantitative risk analysis of carcinogens.

Advantage:

No need for conservative species-species extrapolation, exposure representative for those of target population.

Disadvantage:

Results often hampered by insufficient exposure assessment, other methodological shortcomings or too large random variations between study results.

The <u>MOE</u> and <u>linear extrapolation</u> approaches combine information on human exposure and the same dose descriptors. Both indicate levels of concern and allow ranking between various exposures to such agents.

- For risk communication the MOE is seen preferable.

- For prioritisation of measures, both, the MOE approach and the linear extrapolation from a dose descriptor e.g. the BMDL10 or the T25 are applicable.

- For cost-benefit analysis the linear extrapolation is needed, because it requires likely units of life or ecology under certain exposure conditions.

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Conclusions II

Application of the <u>TTC</u> principle is recommended when appropriate. When exposures are below the relevant TTC level, testing or regulations can be avoided.

The <u>ALARA</u> approach is a valuable measure to minimize exposure to genotoxic and carcinogenic substances. It is not applicable for risk assessment, and it does not provide a basis for setting priorities for action.

Conclusions III

The structural diversity of chemicals and the variability of interactions in biological systems, make it unlikely that (Q)SAR models will achieve sufficient certainty in predicting carcinogenicity. However, (Q)SAR software programs provide the possibility of cost effective screening of chemicals for possible carcinogenicity.

Results of epidemiological studies are always preferred. However, only few are applicable to quantify a carcinogenic risk. Studies are often hampered by insufficient exposure assessment and other methodological shortcomings or too large random variations between study results.

Genotoxic effects can be assessed both *in vitro* and *in vivo*.

For basic *in vitro* testing, assays capable of identifying both gene mutations and chromosomal mutations are employed. Most *in vitro* assays, particularly those detecting DNA breakage and associated chromosomal effects, have low specificity and, consequently, limited predictivity with respect to the carcinogenic potential of agents.

Therefore, demonstration of a genotoxic activity *in vitro* requires further studies *in vivo*.

The follow-up testing *in vivo* assay(s) should be case-by-case considering the toxicokinetics (ADME) of the agent, and the possible genetic endpoint.

In general, the micronucleus assay is recommended as first *in vivo* test. It shows good positive prediction for rodent carcinogenicity and detects both chromosome and genome mutations.

In specific cases, when other organs or tissues than the bone marrow are potential targets, tests to detect such local genotoxic effects are required. These include tests for the induction of DNA repair synthesis (UDS) or DNA strand breaks.

Conclusion

Each compound requires a case-by-case approach taking into consideration all information on the toxicokinetics, toxicodynamics and mode of action to evaluate weight of evidence to justify categorisation on the basis of *in vitro* and *in vivo* studies without appropriate long term studies. Such information is rarely available.

Quantification of the carcinogenic risk is not possible solely on the basis of data from positive *in vitro* and *in vivo* genotoxicity tests.

Threshold for genotoxic carcinogens?

EFSA (2005) concluded that the current understanding of cancer biology suggests levels of exposure to genotoxic and carcinogenic substances below which cancer incidence is not increased.

So far, exposure to even very small amounts of a chemical is considered to result in additional risk.

This low-dose linearity is supported by the linear dose-response for DNA-adduct formation. However, non-linearity in the low-dose range is observed for mutations, relevant in the process of tumour formation. Existence of cellular defence mechanism support EFSA's conclusion.

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Threshold for genotoxic carcinogens?

The contribution of the different cellular defence mechanisms and the dose dependence of their responses are insufficiently understood. Research is needed to elucidate the rate limiting parameters that trigger the defence mechanisms, to permit determination of the onset and saturation of such counterbalancing reactions.

Better understanding is of regulatory importance. A scientifically defendable threshold concept for genotoxic carcinogens will allow identification of NOEL and by that health based exposure limits for genotoxic carcinogens.

Research requirements:

- Development of tools to assess the response of cellular targets to the genotoxic insult at low dose

- Comparison of dose-response of several interrelated biomarkers focusing on genotoxic carcinogens

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Final Conclusion

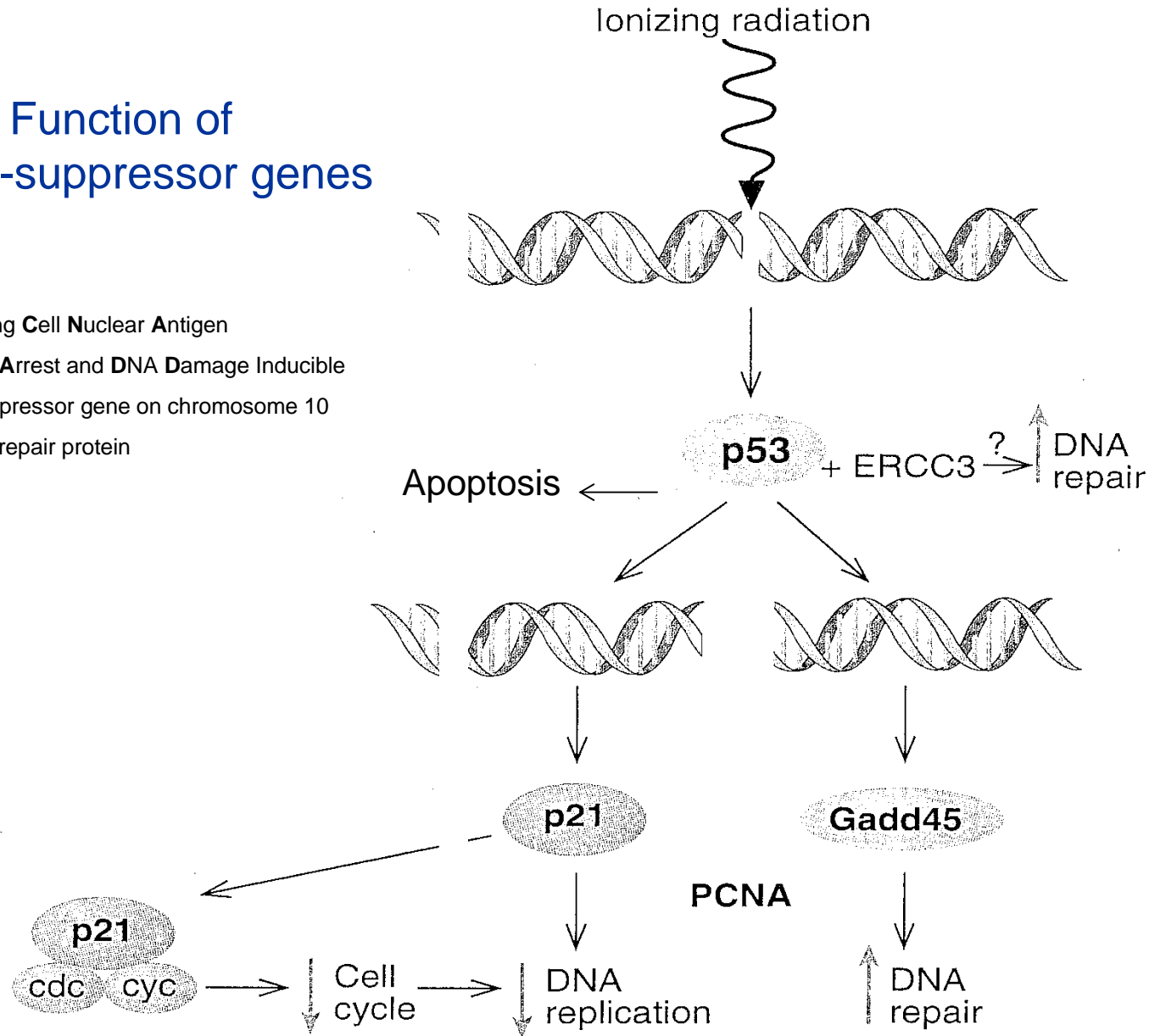The MOE and the linear extrapolation using the same dose descriptor are preferable to the linearised multistage model.

The MOE avoids calculation of cancer incidences at certain exposures.

In vitro/in vivo genotoxicity data are not sufficiently predictive for carcinogenicity and inappropriate for RA.

No Effect Levels for genotoxic carcinogens are likely, although no tools for determination are available.

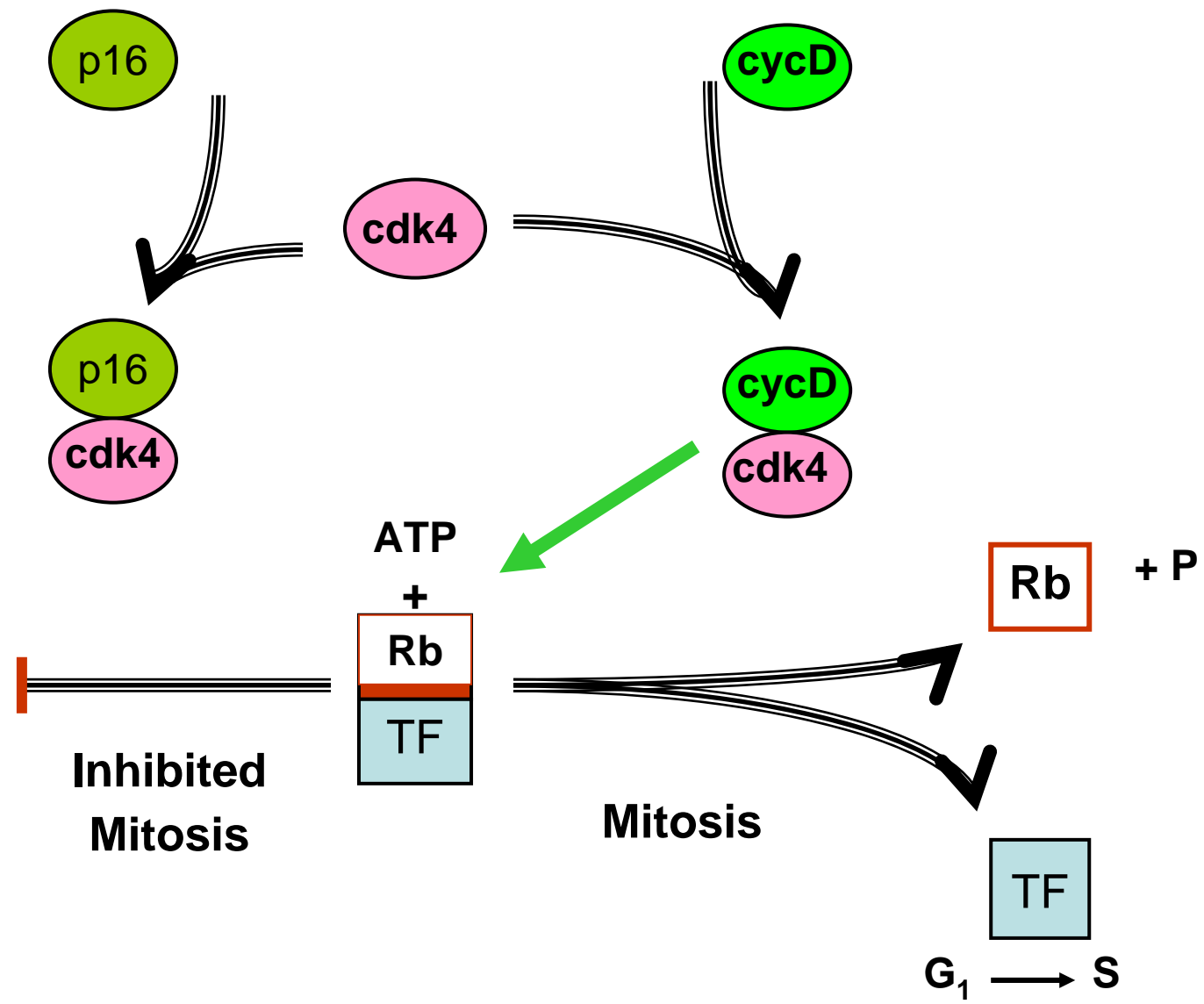Function of Tumor-suppressor genes

Ionizing radiation

PCNA: Proliferating Cell Nuclear Antigen

Gadd 45: Growth Arrest and DNA Damage Inducible

PTEN: Tumor suppressor gene on chromosome 10

ERCC3: Excision repair protein

p53 + ERCC3 →?→ DNA repair

Apoptosis ←

p21

Gadd45

PCNA

p21 cdc cyc → Cell cycle → DNA replication

DNA repair

p16 inhibits mitosis

The CyclinD-Cdk4-complex stimulates cell proliferation by phosphorylating the Retinoblast-oma (Rb)-Protein-TF-Complex.

p16 Protein inhibits cdk4-Binding to cycD thereby blocking mitosis.

p16

cycD

cdk4

p16
cdk4

cycD
cdk4

ATP
+
Rb
TF

Rb   + P

Inhibited Mitosis

Mitosis

TF

$G_1 \longrightarrow S$

THANK YOU