

REGULATED PRODUCTS SAFETY ASSESSMENT

Safety Assessment on the Safety and Efficacy of an Additive of Chromium Propionate (KemTRACE Chromium) as a Feed Additive for All Growing Poultry Species (RP634)

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Keywords: Regulated products, Feed additives, Safety assessment

<https://doi.org/10.46756/001c.121319>

Journal of Food Standards

An application was submitted to the Food Standards Agency in March 2021 from Kemin ('the applicant') for the new authorisation of an additive (KemTRACE Chromium) containing chromium propionate, under the category 'zootechnical' additive, functional group 'other zootechnical additives'. The additive is proposed to be used at an inclusion rate of 0.2 – 0.4 mg/kg of complete feed (12% moisture) for all growing poultry.

The Advisory Committee on Animal Feedingstuffs (ACAF) was asked to review the dossier and the supplementary information submitted by the Applicant, and to advise the Food Standards Agency and Food Standards Scotland (FSA/FSS) in evaluating the dossier.

The FSA/FSS concluded, based on the ACAF's advice, that the additive was correctly characterised, and that it is stable for 24 months at 25°C at 60% humidity, for 6 months as part of a premixture. Homogeneity in feed was also demonstrated. The mixture was pelleted at 90°C, but retention time was not given by the applicant, therefore no conclusion could be drawn on pelleting stability. No further concerns were raised for the identity section, Section II, of the dossier.

The proposed level of inclusion in feed is safe for the target species, but no margin of safety could be determined.

No conclusion could be drawn on the prenatal developmental toxicity potential of the additive, and therefore, on the safety of the additive for consumers. The additive is non-genotoxic, and no concerns about its metabolism were raised, however, no conclusion could be drawn on the applicant's proposed 0-hour withdrawal period, given the impossibility to conclude on the prenatal developmental toxicity potential of the additive.

The active substance is non-irritant to the skin, however another major ingredient, propionic acid, is corrosive to skin and eyes, and the additive should be considered as such. As the active ingredient is corrosive to skin and eyes and it may be sprayed onto the feed, it would be prudent to regard it as potentially harmful by inhalation. The additive is not a skin sensitizer.

The additive poses an acceptable risk to the environment.

Chromium propionate has the potential to be efficacious for all growing poultry at the maximum proposed dose of 0.4 mg/kg of complete feed with 12% moisture.

The views of ACAF have been taken into account in this safety assessment which represents the opinion of the FSA/FSS.

1. Introduction

The FSA/FSS have undertaken a safety assessment for a feed additive (KemTRACE Chromium, Kemin, 34 Botanic Road, Southport, Merseyside, UK, PR9 7NG), consisting of chromium propionate under Assimilated Regulation (EC) No 1831/2003 (EC, 2003) for a new authorisation under the category of 'zootechnical additives' and functional group 'other zootechnicals' for its use in all growing poultry. To support the safety assessment, the AFFAJEG and the ACAF provided advice to the FSA/FSS as outlined in this document.

In line with Article 8 of 1831/2003, the assessment has considered whether the feed additive complies with the conditions laid down in Article 5, including: safety considerations for human, animal and environmental health; efficacy of the additive for its intended effect; potential impairment of the distinctive features of animal products. This, and the guidance put in place by the European Food Safety Authority (EFSA) for the evaluation of feed additive applications, has formed the basis and structure for the assessment.

The dossier was evaluated by the Animal Feed and Feed Additives Joint Expert Group (AFFAJEG) at its July 2022 meeting, after which a request for further information was communicated to the applicant. The applicant's response to this request and subsequent requests were evaluated by AFFAJEG's successor body, the Advisory Committee on Animal Feedingstuffs (ACAF) at its February 2023, July 2023 and October 2023 meetings.

The views of ACAF have been taken into account in this safety assessment which represents the opinion of the FSA/FSS.

From this point on in the document, conclusions made by AFFAJEG or ACAF will be referred to as ACAF, as this was the Committee that concluded on the additive.

Table 1. Table showing products included in this assessment

Title	Product type	Intended use/s	Intended species or categories of animals
Chromium propionate (KemTRACE Chromium)	Feed additive	Zootechnical additive	All growing poultry

2. Assessment

2.1. Section II: Identity, characterisation and conditions of use

The additive's active substance is chromium propionate containing a 7-10% of trivalent chromium [Cr(III)] in liquid form. The applicant provided data from several batches supporting the identification values outlined below ([Table 2](#)):

Table 2. Identity table for the additive, chromium propionate

Composition	
Chromium propionate	29-32 % w/w
Propionic acid	37 % w/w
Sodium propionate	14-17 % w/w
Water	15 % w/w
Propylene glycol	2 % w/w
Appearance	
Green/brown liquid	
Chemical-physical specifications	
Specific gravity	1.2 – 1.25
Viscosity	<2000 cP
Vapor pressure (25°C and 40°C)	<7 kPa
Impurities	
Arsenic	<0.1 mg/kg
Lead	<0.21 mg/kg
Cadmium	<0.02 mg/kg
Mercury	<0.01 mg/kg
Fluorine	<40 mg/kg
PCDD/Fs	0.11 ± 0.022 pg/g
DLPCBs	0.09 ± 0.022 pg/g
PCDD/Fs and DLPCBs	0.20 ± 0.044 pg/g

The Committee discussed the challenge of measuring the concentration of the additive in feeds and premixes, and concluded it would be very difficult to differentiate the chromium concentrations naturally occurring in feed from that of the additive. After a request by FSA, the applicant provided further information to show the accreditation of the laboratories conducting the analyses for methods of analysis, impurities and physico-chemical properties of the additive. The applicant also clarified that the additive is intended to be commercialised as the additive on its own, or in the form of a premix, both for adding to feed.

The additive was shown to be stable for 24 months at 25°C at 60% humidity, for 6 months as part of a premixture. Homogeneity in feed was also demonstrated. No interactions with amino acids in feed were noted. The mixture was pelleted at 90°C, but retention time was not given by the applicant, therefore no conclusion could be drawn on pelleting stability.

The proposed conditions of use of the additive are described in [Table 3](#):

Table 3. Conditions of use of chromium propionate proposed by the Applicant

Proposed mode of use in animal nutrition				
Additive	Chromium propionate			
Registration number/ EC No/No	4dxx			
Category(-ies) of additive	4. Zootechnical feed additive			
Functional group(s) of additive	d. Other			
Description				
Description	Chemical formula	Purity criteria	Method of analysis	
Liquid preparation of organic chromium 7-10 %	C ₉ H ₁₅ CrO ₆	Cr(VI) ≤ 0.1 mg/kg Cr(III) 7-10%	UHPLC-HRMS and ICP-AES	
Trade name (if appropriate)		KemTRACE Chromium		
Name of the holder of authorisation (if appropriate)		Kemin Europa N.V.		
Conditions of use proposed by the applicant				
Species or category of animal	Min-max Age	Min. content	Max. content	Withdrawal period
		Organic chromium in complete feed with a moisture content of 12%.		
All growing poultry	--	0.2 mg/kg	0.4 mg/kg	--

2.1.1. Conclusions on Section II

The ACAF concluded the additive was correctly characterised, and that it is stable for 24 months at 25°C at 60% humidity, for 6 months as part of a premixture. Homogeneity in feed was also demonstrated. The mixture was pelleted at 90°C, but retention time was not given by the applicant, therefore no conclusion could be drawn on pelleting stability. No further concerns were raised for Section II of the dossier.

2.2. Section III: Safety

2.2.1. Safety for the target species

One tolerance study in chickens for fattening was presented to evaluate the safety of the additive for the target species. No mortality was observed. No significant differences were identified for performance parameters, including the overdose group. Haematological and histological results were considered normal. Results are presented in [Table 4](#).

Table 4. Tolerance study on broilers: Summary of performance characteristics

		Body weight (g/bird/35 d)	Weight gain (g/bird/35 d)	Feed intake (g/bird/35 d)	FCR (-)
Control	Control	2202.9 ±12.4 ^b	2160.5 ±12.3 ^b	3229.7 ±31.9 ^a	1.50 ±0.02 ^a
T 1	0.4 mg Cr/kg	2364.3 ±18.3 ^a	2321.8 ±18.2 ^a	2321.8 ±18.2 ^a	1.37 ±0.02 ^b
T 2	4 mg Cr/kg	2362.4 ±22.1 ^a	2319.9 ±21.9 ^a	2319.9 ±21.9 ^a	1.37 ±0.03 ^b
SEM		5.204	5.161	5.161	0.01

^{a,b} – means with different letters in the same row are significantly different at $p < 0.05$; Tukey test after a significant one-way-Anova ($p < 0.05$). SEM: Standard Error of Mean.

The study was not conducted to GLP and deviated slightly from Guidance recommendations. The Committee noted several shortcomings on the study reporting, including evidence of the research organisations quality system, processes or institute protocols. This information was given by the applicant after request by FSA and reviewed by ACAF, after which it was concluded that the study showed the additive is safe at the level of inclusion of 0.4 mg Cr/kg of complete feed. A reduction in the weight of several organs was noted in both dosing groups, which could not be ruled out as a potential dose-dependent side effect. Based on this observation, no margin of safety could be established in this study.

Additional evidence was provided by the applicant in the form of a peer-reviewed published study using doses of 0.4 and 2 mg Cr/kg of complete feed, conducted to GLP but not following Guidance recommendations. The study results did not report an improvement in performance markers, showing slightly lower mean final body weights and body weight gain in males for the highest dose groups in growing and finishing diets. The ACAF did not consider these to be adverse effects, and concluded that the study showed that the additive is tolerated by the target species at 0.4 mg/kg concentration.

2.2.2. Safety for the consumer

The applicant provided a battery of toxicological studies to support the safety for the consumer section of the dossier. A summary of the tests and results can be found in [Table 5](#):

Table 5. Consumer safety: Chromium propionate summary of studies

Test	Species	OECD	GLP	Results
Metabolic and residue studies				
Metabolic study	<i>In vitro</i>	N/A	No	<ul style="list-style-type: none"> • Cr(III) stable upon digestion. • Levels of toxic Cr(VI) below detection limit (<40 µg/L). • Uptake of Cr into cells was very low
Residue study	Chickens	N/A	No	<ul style="list-style-type: none"> • No effect in Cr concentrations in chicken muscle or skin tissues.
Genotoxicity studies				
Bacterial reverse mutation assay	<i>In vitro</i>	471	Yes	<ul style="list-style-type: none"> • No increase in the mean number of revertants per plate • Results did not indicate mutagenicity
Mammalian cell micronucleus test	<i>In vitro</i>	487	Yes	<ul style="list-style-type: none"> • No increase in the micronuclei frequency in cultured human peripheral blood lymphocytes during short/long term exposure • Results did not indicate mutagenicity
Mouse lymphoma assay	<i>In vitro</i>	476	Yes	<ul style="list-style-type: none"> • Evaluated as negative, interpreted as not having the ability to induce mutations • Results did not indicate mutagenicity
Mammalian erythrocyte micronucleus test	Mice	474	Yes	<ul style="list-style-type: none"> • Oral gavage administration • No signs of clinical toxicity up to 2000 mg/kg • Not cytotoxic to the bone marrow • Absence of clinical signs and bone marrow cytotoxicity suggests absence of micronuclei in the study cannot be interpreted as a negative result
Repeat dose toxicity studies				
Sub-chronic oral toxicity (28 days)	Rats	407	Yes	<ul style="list-style-type: none"> • NOAEL of study agreed to be highest dose tested of 1000 mg/kg
Sub-chronic oral toxicity (90 days)	Rats	408	Yes	<ul style="list-style-type: none"> • Clinical signs of greater severity than at lower dose levels noted at dose of 1000 mg/kg bw/day • NOAEL of study claimed to be 500 mg/kg bw/day • Clinical signs, consistent with nasal irritation were observed at and above 250 mg/kg bw/day, and a dose-related

Test	Species	OECD	GLP	Results
				<p>increase in serum activity of aspartate aminotransferase (AST) was observed at and above 250 mg/kg bw/day.</p> <ul style="list-style-type: none"> • ACAF considered the LOAEL to be 250 mg/kg bw/day, with no NOAEL identified.
Chronic toxicity study (6-8 months)	Rats	452	Yes	<ul style="list-style-type: none"> • Well-tolerated and did not produce any effect on specific organs/systems • NOAEL of study found to be 200 mg/kg bw/day
Reproduction toxicity studies				
Two-Generation Reproductive Toxicity Study	Rats	416	Yes	<ul style="list-style-type: none"> • No treatment-related effects on integrity of the male and female reproductive systems • No neonatal morbidity or mortality • Study claimed no effect on prenatal and postnatal development of F1 and F2 offspring; however, this study design only provided limited information on prenatal developmental toxicity, including numbers of live births, stillbirths and the presence of gross anomalies, and did not include examination of fetuses for soft tissue and skeletal malformations and does not allow us to conclude on potential developmental toxicity. • NOAEL of study found to be 600 mg/kg bw/day

The three *in vitro* tests (bacterial reverse mutation, mammalian cell micronucleus and mouse lymphoma) were interpreted by the Committee as being negative. The *in vivo* micronucleus test showed no signs of clinical toxicity at doses up to 2000 mg/kg and no signs of cytotoxicity to the bone marrow. Due to this, the study results showing an absence of micronuclei cannot be considered to provide evidence on the absence of genotoxicity *in vivo* as systemic exposure was not demonstrated. Three further toxicological studies were evaluated, a sub-chronic oral toxicity in rats (28 days), a sub-chronic oral toxicity in rats (90 days) and a chronic toxicity study (6-12 months). Clinical signs were shown in the sub-chronic study (90 days) at all dose levels tested (250 – 1000 mg/kg bw/day), which were of greatest severity at the 1000 mg/kg dose, and there was a dose-related increase in serum aspartate aminotransferase (AST) in this study. No other dose-related clinical signs were reported in the other sub-chronic and chronic toxicity studies.

The study authors proposed a NOAEL of 1000 mg/kg bw/day for the 28-day subchronic toxicity study, 500 mg/kg bw/day for the 90-day subchronic toxicity study, and 200 mg/kg bw/day for the chronic toxicity study. Members considered that the LOAEL in the 90-day subchronic toxicity study was possibly 250 mg/kg bw/day, with no NOAEL identified, but agreed that the overall NOAEL from the repeat-dose toxicity studies was 200 mg/kg bw/day, as shown in the chronic toxicity study. Members agreed that no significant adverse effects were identified in the two-generation reproduction toxicity study, for which authors proposed a NOAEL of 600 mg/kg bw/day. The Committee concluded that, taken together, the NOAEL for the four studies should be 200 mg/kg bw/day.

Based on the conclusions from the in vitro genotoxicity tests, the Committee concluded that the additive is non-genotoxic.

The Committee challenged the decision by the applicant to not submit prenatal developmental studies (PNDT). After two requests for further information by the FSA, the applicant referred to other literature studies to rationalise the absence of PNDT studies. This was concluded to not be acceptable by the ACAF, as several literature references pointed to the embryotoxicity potential of the compound, and the literature studies presented were not acceptable as substitutes for a PNDT study. The applicant chose to not carry out a PNDT study, and therefore the Committee could not conclude on the prenatal developmental toxicity potential of the additive, and therefore a safe level of exposure for all consumers could not be identified.

The Committee agreed that the other components of the additive propionic acid, sodium propionate and propylene glycol, leave minimal residues and would not pose a risk to consumers. No further causes for concern were raised for the metabolism of the additive in the organism of the target species, however, the ACAF could not conclude on the applicant's proposed 0-hour withdrawal period, given the impossibility to conclude on the prenatal developmental toxicity potential of the additive.

2.2.3. Safety for the user

The applicant presented the following tests carried out using the active substance, chromium propionate, to evaluate the safety of the additive for the user/worker:

- Local lymph node assay for skin sensitisation to GLP, following OECD 429 protocol.
- Two acute dermal irritation/corrosion studies for dermal irritation to GLP, following OECD 404 protocol.

- Acute eye irritation/corrosion study to GLP, following OECD protocol 405.

The Committee confirmed the applicant's conclusions that the active substance is non-irritant to the skin, however propionic acid is corrosive to skin and eyes, and is present in the additive at a concentration of 14-17 % w/w. Therefore, the additive should be considered as corrosive to skin. The additive is not a skin sensitizer. Given the additive is corrosive to skin and eyes, it is likely to also be harmful through inhalation if sprayed directly onto the feed.

2.2.4. Safety for the environment

The applicant carried out a Phase I environmental risk assessment showing that concentration of chromium in the environment would not be significantly affected by the use of the additive at the proposed conditions of use. The ACAF concluded that the additive poses an acceptable risk for the environment.

2.2.5. Conclusions on safety

The ACAF concluded that the proposed level of inclusion in feed is safe for the target species, but no margin of safety could be determined.

The additive is non-genotoxic, and no concerns about its metabolism were raised by the Committee, however, the ACAF could not conclude on the applicant's proposed 0-hour withdrawal period, given the impossibility to conclude on the prenatal developmental toxicity potential of the additive.

The ACAF could not conclude on the prenatal developmental toxicity potential of the additive.

The active substance is non-irritant to the skin, however another major ingredient, propionic acid, is corrosive to skin and eyes, and the additive should be considered as such. As the active ingredient is corrosive to skin and eyes and it may be sprayed onto the feed, it would be prudent to regard it as potentially harmful by inhalation. The additive is not a skin sensitizer.

The additive poses an acceptable risk to the environment.

2.3. Section IV: Efficacy

The applicant presented three efficacy studies and a meta-analysis to show evidence of efficacy for chromium propionate in growing poultry. The applicant concluded that growth performance, feed conversion, carcass yield, breast and leg meats of chickens for fattening were improved by supplementation with chromium propionate.

The Committee questioned the validity of the study results, noting several shortcomings in study design, study reporting and absence of quality assurance certifications. Further information was provided after request by FSA, which was not deemed as sufficient to demonstrate the validity of the studies. The FSA requested the input of an internationally recognised specialist in poultry nutrition, Robert Pym, who confirmed that, although some of the values reported were very unusual, the trials should still be considered valid. The ACAF evaluated the specialist's report, accepted the validity of the studies and reviewed the efficacy data, concluding that the additive had the potential to be efficacious when included in feed at the maximum proposed dose of 0.4 mg/kg of complete feed with 12% moisture. Efficacy was not demonstrated at the lower inclusion rate of 0.2 mg/kg.

2.3.1. Conclusions on efficacy

The ACAF concluded that chromium propionate has the potential to be efficacious for all growing poultry at the maximum proposed dose of 0.4 mg/kg of complete feed with 12% moisture.

Table 6. Conditions of use of chromium propionate proposed by the Committee

Conditions of use supported by the Committee				
Species or category of animal	Min-max Age	Min. content	Max. content	Withdrawal period
		Organic chromium in complete feed with a moisture content of 12%.		
All growing poultry	--	0.4 mg/kg	0.4 mg/kg	Cannot conclude

3. Analytical methods evaluation

Conclusions on the analytical methods are presented here as an extract from the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of the Analysis for Chromium Propionate (EURL-FA, 2020):

"For the quantification of the chromium propionate content in the feed additive the Applicant submitted two single-laboratory validated methods, namely a method based on liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) and a method based on liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The LC-MS/MS method was further verified and the following performance characteristics were obtained for the quantification of the chromium propionate content in the feed additive in the frame of the validation and verification studies: a relative standard deviation for repeatability (RSDr) ranging from 2.0 to 7.2 %, a relative standard deviation for

intermediate precision (RSDip) ranging from 5.5 to 7.9 % and a recovery rate (Rrec) ranging from 91 to 103 %. Based on the acceptable performance characteristics available, the EURL recommends for official control the single-laboratory validated and further verified method based on LC-MS/MS for the quantification of the chromium propionate content in the feed additive. For the quantification of the chromium propionate content in premixtures and feedingstuffs the Applicant submitted the above mentioned methods based on LC-HRMS and LC-MS/MS after an appropriate sample preparation. However, the Applicant did not provide the EURL with proper validation and/or verification data when applying the LC-HRMS and/or LC-MS/MS methods for the quantification of chromium propionate in premixtures and feedingstuffs. Based on the available performance information, the EURL is not able to recommend for official control the above mentioned methods based on LC-HRMS or LC-MS/MS for the quantification of the chromium propionate content in premixtures and feedingstuffs.

For the quantification of the total chromium content in the feed additive the Applicant submitted a single-laboratory validated and further verified method based on inductively coupled plasma-atomic emission spectrometry (ICP-AES). The following performance characteristics were obtained for the quantification of the total chromium content in the feed additive in the frame of the validation and verification studies: a RSDr ranging from 0.3 to 0.9 %, a RSDip ranging from 0.9 to 1.1 % and a Rrec of 100 %. Based on the acceptable performance characteristics available, the EURL recommends for official control the single-laboratory validated and further verified method based on ICP-AES for the quantification of the total chromium content in the feed additive (chromium propionate). For the quantification of the organic chromium content in mineral-vitamin premixtures and feedingstuffs the Applicant proposed in-house methods based on ICP-AES and/or ICP-MS. Non-acceptable recoveries (lower than 60 %) were reported for an average organic chromium content in the analysed samples of premixtures and feedingstuffs. Based on the available data, the EURL is not able to recommend for official control the proposed methods based on ICP-AES or ICP-MS, neither any other method for the quantification of the organic chromium content in premixtures and feedingstuffs."

FSA/FSS accepts the EURL analytical method evaluation reports. FSA/FSS determined the analytical method as appropriate for official controls for this feed additive.

4. Conclusions

The ACAF concluded the additive was correctly characterised, and that it is stable for 24 months at 25°C at 60% humidity, for 6 months as part of a premixture. Homogeneity in feed was also demonstrated. The mixture was pelleted at 90°C, but retention time was not given by the applicant, therefore no conclusion could be drawn on pelleting stability. No further concerns were raised for Section II of the dossier.

The ACAF concluded that the proposed level of inclusion in feed is safe for the target species, but no margin of safety could be determined.

The ACAF could not conclude on the prenatal developmental toxicity potential of the additive, and therefore, on the safety of the additive for consumers. The additive is non-genotoxic, and no concerns about its metabolism were raised, however, the ACAF could not conclude on the applicant's proposed 0-hour withdrawal period, given the impossibility to conclude on the prenatal developmental toxicity potential of the additive.

The active substance is non-irritant to the skin, however another major ingredient, propionic acid, is corrosive to skin and eyes, and the additive should be considered as such. As the active ingredient is corrosive to skin and eyes and it may be sprayed onto the feed, it would be prudent to regard it as potentially harmful by inhalation. The additive is not a skin sensitizer.

The additive poses an acceptable risk to the environment.

The ACAF concluded that chromium propionate has the potential to be efficacious for all growing poultry at the maximum proposed dose of 0.4 mg/kg of complete feed with 12% moisture.

The FSA/FSS agree with the conclusions reached by the ACAF. FSA/FSS accepts the EURL analytical method evaluation reports. FSA/FSS determined the analytical method as appropriate for official controls for this feed additive.

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Abbreviations

Acronym	Definition
ACAF	Advisory Committee on Animal Feedingstuffs

Acronym	Definition
AFFAJEG	Animal Feed and Feed Additives Joint Expert Group
CAS	Chemical Abstracts Service
cP	Centipoise
Cr	Chromium
EFSA	European Food Safety Authority
EURL	European Union Reference Laboratory
FSA	Food Standards Agency
FSS	Food Standards Scotland
GLP	Good Laboratory Practices
ICP-AES	Inductively coupled plasma-atomic emission spectrometry
ICP-MS	Inductively coupled plasma mass spectrometry
kPa	Kilopascal
LC-HRMS	Liquid chromatography coupled to high resolution mass spectrometry
LC-MS/MS	Liquid chromatography coupled to tandem quadrupole mass spectrometry
NOAEL	No observed adverse effect level
PCDD/Fs	Polychlorinated dibenzo-p-dioxins and dibenzofurans
DLPCBs	Dioxin-like polychlorinated biphenyls
OECD	Organisation for Economic Co-operation and Development
pg	Picogram
PNDT	Prenatal developmental toxicity
Rrec	Recovery rate

Acknowledgements

With thanks to the members of the ACAF during the course of the assessment, who were: Professor Nicholas Jonsson, Professor Emily Burton, Martin Briggs, Professor Katrina Campbell, Professor Matthew Fisher, Hannah Kane, Susan MacDonald, Dr. Oonagh Markey, Christine McAlinden, Dr. Donald Morrison, Derek Renshaw, Dr. Michael Salter, Dr. Adam Smith, Christel Wake, Dr. Helen Warren and Dr. Nick Wheelhouse.



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